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## NATURAL CARBONATES OF CALCIUM AND MAGNESIUM IN RELATION TO THE CHEMICAL COMPOSITION, BAC- TERIAL CONTENTS, AND CROP-PRODUCING POWER OF TWO VERY ACID SOILS

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### INTRODUCTION

Agricultural limestones so frequently contain large quantities of magnesium that the question of the relative values of calcium and magnesium carbonates as neutralizers of soil acidity is of great practical importance. Many instances where magnesium has had detrimental effects on plant growth have been reported, as have also other instances where magnesium limestones have produced greater crop growth than did pure calcium limestones. The literature on the subject is very extensive and has been fully reviewed by others (3, 4, 5, 6).<sup>2</sup>

The present paper reports results of pot and laboratory tests on two very acid soils of distinctly different types. The data were all obtained from experiments conducted under controlled moisture conditions with natural carbonates of high purity. The calcite used analyzed 56 per cent calcium oxid and 0.1 per cent magnesium oxid. The dolomite contained 30.4 per cent calcium oxid and 20.5 per cent magnesium oxid. The magnesite contained 0.12 per cent calcium oxid and 46.2 per cent magnesium oxid. No basic or artificial carbonates were used in the experiments reported.

### SOILS USED

The analyses of the soils used, an acid silty clay very low in organic matter content and an acid black peaty sand high in organic matter content, are given in Table I.

The two soils are shown to be of quite different composition. The variation between the nitrogen and humus determinations is large. Both the Hopkins<sup>3</sup> potassium-nitrate and the Jones<sup>4</sup> calcium-acetate

<sup>1</sup> Resigned Nov. 1, 1918.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 125.

<sup>3</sup> WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 127 (rev.), 272 p., 13 figs., 1908. Reprinted 1912.

<sup>4</sup> JONES, C. H. METHOD FOR DETERMINING THE LINE REQUIREMENT OF SOILS. *In* Jour. Assoc. Off. Agr. Chem., v. 1, no. 1, p. 43-44. 1915.

method show the soils as quite acid. That the substances in the soil which react with the potassium nitrate and calcium acetate are not entirely the same is plainly shown in the different acidity results obtained by the two methods. Neither soil can be said to contain a normal amount of calcium, and the magnesium of both is more than twice the calcium content.

TABLE I.—Analyses of soils used

Determinations <sup>a</sup>	Yellow clay.	Black sand.
	<i>Per cent.</i>	<i>Per cent.</i>
Volatile matter.....	3.57	10.13
Potassium oxid (K <sub>2</sub> O).....	.27	.21
Calcium oxid (CaO).....	.18	.10
Magnesium oxid (MgO).....	.40	.23
Manganese oxid (Mn <sub>2</sub> O <sub>4</sub> ).....	.08	.04
Ferric oxid (Fe <sub>2</sub> O <sub>3</sub> ).....	3.68	1.04
Aluminum oxid (Al <sub>2</sub> O <sub>3</sub> ).....	4.68	3.09
Phosphorus oxid (P <sub>2</sub> O <sub>5</sub> ).....	.05	.10
Sulphate (SO <sub>3</sub> ).....	.12	.11
Residue.....	87.76	85.50
Nitrogen.....	.07	.40
Humus (acid) <sup>b</sup> .....	.73	5.72
Humus <sup>c</sup> .....	.70	4.96
Hygroscopic moisture.....	1.50	1.90
Acidity:		
Potassium-nitrate method.....	<i>Pounds<sup>d</sup></i>	<i>Pounds<sup>d</sup></i>
Calcium-acetate method.....	2,730.00	1,260.00
	2,940.00	5,310.00
	<i>Gm.<sup>e</sup></i>	<i>Gm.<sup>e</sup></i>
Water-holding capacity.....	48.6	67.1

<sup>a</sup> WILEY, H. W. OP. CIT.

<sup>b</sup> Ammonia soluble without previous washing with dilute hydrochloric acid.

<sup>c</sup> Washed with hydrochloric acid, digested with ammonia, filtered, and refiltered till clear.

<sup>d</sup> Pounds of calcium carbonate required to neutralize one million pounds of soil.

<sup>e</sup> Grams of water per 100 gm. of dry soil.

#### GREENHOUSE TESTS

Pot tests were conducted in duplicate for all treatments on each soil. The crops in the order in which they were grown were wheat, red clover, and blood turnip beets. The containers for the soil were galvanized iron pots, 9.25 inches in diameter and 11 inches deep, paraffined well on the inside. One-inch galvanized iron tubes, connected with an arch at the bottom of the pot (both well paraffined), were provided for aeration and the addition of water. The seed was selected with care, planted uniformly, and the resulting plants were kept under good conditions for their development. The soil was kept at optimum moisture content by weighing the pots at regular intervals and replenishing the water lost with distilled water. The various soil treatments employed and the crop yields are given in Table II.

Table II shows that the 4,000-pound applications of calcite, magnesite, and dolomite gave similar results on both soils. The differences between the calcite, magnesite, and dolomite increases were small for the wheat and clover, while the magnesium carbonate gave much larger increases with the beets.

TABLE II.—Yields of air-dry wheat and clover and undried blood turnip beets on acid soils treated with natural carbonates of calcium and magnesium

Pot No.	Treatment per million pounds of soil. <sup>a</sup>	Average yields in grams per pot.							
		Yellow clay.				Black sand.			
		Wheat. Clover.		Beets.		Wheat. Clover.		Beets.	
				Tops.	Roots.			Tops.	Roots.
3	Control.....	44.0	2.0	0.0	0.0	1.5	3.5	0.0	0.0
5	4,000 pounds calcite....	65.5	18.5	36.0	18.0	31.5	12.5	23.0	13.5
10	4,000 pounds magnesite..	64.0	16.0	53.0	33.5	29.0	8.5	51.0	23.0
9	4,000 pounds dolomite...	62.5	20.0	44.5	22.5	35.5	11.5	33.0	21.0
13	12,000 pounds calcite....	77.5	15.5	56.5	49.5	51.0	15.0	50.0	21.0
14	12,000 pounds magnesite	71.0	16.5	63.0	51.5	.0	.0	3.0	.5

<sup>a</sup> A basic application of 75 pounds diammonium phosphate and 100 pounds dipotassium phosphate per million pounds of soil was made to each pot. In addition the yellow clay soil received a total of 90 pounds per million of ammonium nitrate, applied in three equal successive applications.

The 12,000-pound applications of calcite and magnesite gave different results on the two soils. The most apparent of these differences were noted in the detrimental effects of the 12,000-pound magnesite application on the black sand and the increased crop yields on the yellow clay due to additional magnesite. While the increases due to heavier applications were not proportionally larger, they checked the results of the 4,000-pound applications. The greatest increases in crop yields for the 12,000—over the 4,000-pound applications of natural carbonates were with the calcite on the black sand. It should be noted that the yield of beets on the black sand, although much increased over that obtained with the 4,000-pound calcite application, is not so large as that obtained with the 4,000-pound treatment of magnesite.

These varying yields with different crops are in accord with the results obtained by Coupin (1) who found that the action of magnesium carbonate was different on different species of plants.

Plate 1 shows the appearance of the various crops grown on the acid yellow clay soil. Pot 4, not discussed in the tables, had an application of one-half as much calcite as pot 5.

Plate 2 shows the appearance of the various crops grown on the acid black sand. Pot 4 had one-half as much calcite as was applied to pot 5. The appearance of the beets in this series would indicate that magnesium was not so harmful to beets as it was to wheat and clover.

At certain stages of growth the wheat growing in the pots treated with magnesite showed a dark green color, as noted by others (5), while the wheat in the calcite-treated pots was a light yellowish green. Although the magnesite caused almost as much wheat increase in all except the 12,000-pound magnesite application on the black sand,

there was at times during the vegetative stage of growth a tendency toward tip burning wherever magnesite was applied. This unfavorable condition, somewhat similar to that observed by Dickson (2), was not noted on the calcite- or dolomite-treated wheat and did not appear to cause permanent injury in any case.

TABLE III.—Soluble salts, nitrates, bacterial contents, acidity, and carbon-dioxide content of two acid soils as affected by natural carbonates of calcium and magnesium

YELLOW CLAY									
Treatment per million pounds of soil.	Soluble salts, parts per million.	Nitrate (NO <sub>3</sub> ) content, parts per million.		Percentage of carbon dioxide.	Acidity, as calcium carbonate needed per million pounds of soil.		Bacterial content, millions per gram of soil.		Ratio of calcium acid to magnesium acid.
		As sampled.	After nitrification.		Hopkins method.	Jones method.	Aerobic.	Anaerobic.	
Control.....	412	Trace.	Trace.	0.02	5,600	8,350	3,077	0.000	1:2.2
4,000 pounds calcite.....	272	0	184	.04	40	1,500	7,605	0.000	1:1.0
4,000 pounds magnesite.....	604	0	355	.04	40	1,250	12,229	3,220	1:3.0
4,000 pounds dolomite.....	(b)	0	.....	.04	80	1,500	.....	.....	1:1.6
12,000 pounds calcite.....	434	0	873	.28	0	1,100	5,244	0.000	1:1.0
12,000 pounds magnesite.....	614	0	842	.25	0	750	9,138	1,133	1:5.3
BLACK SAND									
Control.....	448	350	340	0.03	3,520	13,500	2,813	2,997	1:0.2.3
4,000 pounds calcite.....	280	52	913	.11	80	5,000	10,383	0.099	1:4.1.0
4,000 pounds magnesite.....	360	220	1,000	.10	120	5,000	6,833	0,410	1:0.2.0
4,000 pounds dolomite.....	.....	.....	.....	.10	120	5,000	.....	.....	1:0.2.8
12,000 pounds calcite.....	432	233	1,280	.23	20	1,500	10,017	3,700	3:4.1.0
12,000 pounds magnesite.....	1,320	915	2,544	.23	20	1,000	5,837	625	1:0.2.8

<sup>a</sup> Figures calculated to dry basis.

<sup>b</sup> Blanks in dolomite column indicate that no determinations were made.

Table III gives the results of tests on the soils made 10 months after the experiments were started, when the pots contained growing clover and wheat stubble. The samples were drawn with Noyes' bacteriologists' soil samplers<sup>1</sup> to the full depth of the pots. Nitrates, bacterial numbers, nitrification and soluble salts were determined on the moist samples. Acidity and carbon dioxide were determined on air-dry samples.

Soluble salts were determined by means of the electrical bridge<sup>2</sup> and show the relative quantities of ionized salts in the soil solution. The nitrates were determined by the modified phenol-disulphonic acid method.<sup>3</sup> Nitrification was carried out in tumblers by the beaker method. Carbon dioxide was determined with boiling hydrochloric acid (specific gravity 1.115). Acidity was determined by the Hopkins<sup>4</sup>

<sup>1</sup> NOYES, H. A. SOIL SAMPLING FOR BACTERIOLOGICAL ANALYSIS. *In* Jour. Amer. Soc. Agron., v. 7, no. 5, p. 232-249, fig. 13, pl. 4. 1915.

<sup>2</sup> DAVIS, R. O. E., and BRYAN, H. THE ELECTRIC BRIDGE FOR THE DETERMINATION OF SOLUBLE SALTS IN SOILS. U. S. Dept. Agr. Bur. Soils Bul. 61, 36 p., 7 fig., 2 pl. 1910.

<sup>3</sup> NOYES, H. A. ACCURATE DETERMINATION OF SOIL NITRATES BY PHENOL DISULPHONIC ACID METHOD. *In* Jour. Indus. and Engin. Chem., v. 11, no. 3, p. 213-218. 1919.

<sup>4</sup> WILEY, H. W. OF CIT.

potassium-nitrate and by the Jones<sup>1</sup> calcium-acetate methods. Bacterial counts were the average of counts on five plates made after 10 days' incubation at 20° C. by the method of Noyes and Voigt.<sup>2</sup>

The quantities of salts, as determined by the electric bridge, were greater with the use of the magnesite than with the calcite. This, to a certain extent, illustrates the comparative solubilities of the calcium and magnesium compounds as well as of other soluble salts resulting from the reactions taking place between these carbonates and the soil constituents.

The carbon-dioxid determinations show that the decomposition of the added natural carbonates was not complete in any case at the end of 10 months. The data tend to confirm, however, MacIntire's statement (6) that magnesium carbonates are more readily decomposed than is calcium carbonate.

While each soil (Table I) contained approximately two-parts of magnesium oxid to one part of calcium oxid there was almost twice as much of both calcium and magnesium oxids in the clay soil as in the sandy soil. Six tons of magnesite per million pounds of soil increased the magnesium oxid content by 0.57 per cent. This made the ratios of calcium oxid to magnesium oxid approximately 1 to 8 for the sandy soil and 1 to 5 for the clay soil. The 2-ton application of magnesite which was not injurious made the ratio of calcium oxid to magnesium oxid 1 to 4 for the sandy soil. It might be contended that the sandy soil would have produced crops with a ratio of 1 to 5 between calcium oxid and magnesium oxid, but it must be remembered that in any case only small portions of the total calcium and magnesium were in solution, and it is quite probable that the clay soil would offer more resistance to the injurious action of the magnesium salts than the sand would.

The 12,000-pound application of magnesite gave 1,320 pounds of soluble salts per million of the black sand and only 624 on the yellow clay. Recent tests by one of the writers (unpublished data) have shown that magnesium carbonate increases the solubility of soil constituents more than calcium carbonate does. Soluble magnesium in quantity has long been known to be detrimental to plant and bacterial development. This is in accord with both the high soluble salt content and low aerobic bacteria counts on the black sandy soil with the 12,000-pound application of magnesite.

In a previous paper (7) it has been shown that nitrification occurs in these acid soils and that the amounts of nitrates found in the soils when sampled are largely influenced by the growing crop. The nitrates after incubation are evidence that liming increases the nitrifying power of the soils. In all cases magnesite caused greater nitrification than did

<sup>1</sup> JONES, C. H. OP. CIT.

<sup>2</sup> NOYES, H. A., and VOIGT, Edwin. A TECHNIC FOR THE BACTERIOLOGICAL EXAMINATION OF SOILS. *In Proc. Ind. Acad. Sci.* 1916, p. 172-191, 6 fig. 1917.

calcite. The number of aerobic organisms was increased by liming, which is evidence that in general those conditions that are favorable to plant growth are also favorable to bacterial activity.

On the yellow clay the magnesite caused greater bacterial growth, while on the black sand the calcite caused the greater increases. The bacterial differences between the magnesite and calcite results on these two soils are probably partly due to the relative availability of the plant food present. The magnesite, which increases soluble soil constituents more than calcite, gave the greater increases on the yellow clay—low in available plant food—and the lesser increases on the black sand—relatively richer in available plant food. The heavy application of magnesite unhindered by clay, with which it would form insoluble compounds, caused too high a concentration of soluble salts on the black sand. The black sand, though less compact, was high enough in organic matter to contain normally the more anaerobes.

The crop results do not point to any particular ratio between calcium and magnesium which could be called optimum for either soil or crop. This is in accord with the results of Waynick (8) and others.

It is not possible with the data at hand to determine how much the injurious action of the high magnesite application on the black sand was due to an unfavorable calcium-magnesium ratio and how much was due to the high concentration of soluble magnesium salts; but, in view of the fact that the black sand soil still gave an acid reaction after the heaviest magnesite application, it is evident that the crop injury was not due to alkalinity.

#### SUMMARY

(1) Pot experiments were conducted on two very acid soils of different types, using the natural carbonates, calcite, dolomite, and magnesite, in varying amounts. Wheat, red clover, and blood turnip beets were grown in succession.

(2) After being cropped 10 months under optimum moisture conditions the soils were tested for soluble salts, nitrates, nitrification, carbon dioxide, acidity, and both aerobic and anaerobic bacteria.

(3) Although both soils originally contained twice as much magnesium oxid as calcium oxid, still calcite, dolomite, and magnesite, in both quantities used, produced, with one exception, good crop increases on both soils. The 6-ton application of magnesite on the black sand soil killed the crops.

(4) Good crop increases were obtained with carbonate applications which produced ratios of calcium oxid to magnesium oxid varying from 2:1 to 1:5.3 on the yellow clay soil and from 3.4:1 to 1:4 on the black sand. The 6-ton application on the black sand which caused crop failure gave a ratio of 1 calcium oxid to 7.9 magnesium oxid.

(5) Wheat, red clover, and beets responded differently toward calcium and magnesium carbonates. With the medium applications beets were

benefited more by magnesium carbonate, while wheat and clover gave greater increases with calcium than with magnesium carbonate.

(6) Magnesite in all instances increased the concentrations of soluble salts in the soils more than calcite.

(7) Carbon dioxid determinations showed that the carbonates were not entirely decomposed at the end of one year. The decomposition of the magnesite seems to have proceeded faster than that of the calcite.

(8) Magnesite produced more favorable conditions for nitrification than did calcite.

(9) Magnesite encouraged the multiplication of both aerobic and anaerobic bacteria on the yellow clay soil more than calcite did. On the black sand soil the reverse was true. Calcite increased the bacterial content of the soil more than did magnesite.

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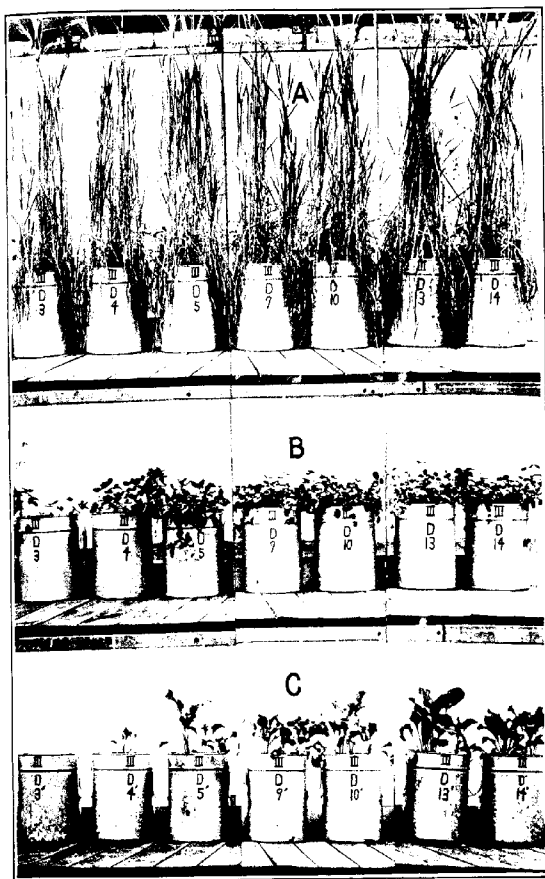


PLATE 1

Pot cultures on acid yellow clay soil:

A.—Wheat; B.—Red clover; C.—Red turnip beets.

SERIES NO.	TREATMENT PER MILLION POUNDS SOIL.
D 3	No carbonates.
D 4	1 ton calcite.
D 5	2 tons calcite.
D 9	2 tons dolomite.
D 10	2 tons magnesite.
D 13	6 tons calcite.
D 14	6 tons magnesite.



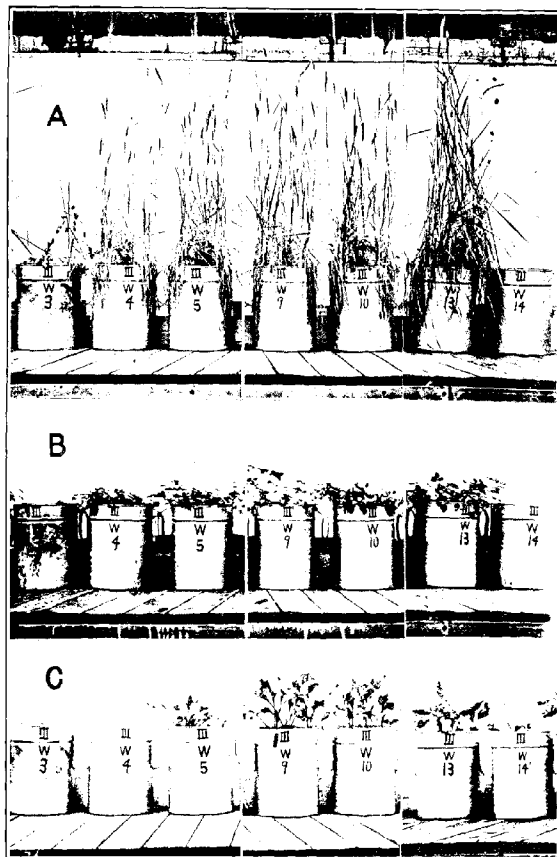


PLATE 2

Pot cultures on acid black sand soil:

A.—Wheat; B.—Red clover; C.—Red turnip beets.

SERIES NO.	TREATMENT PER MILLION POUNDS SOIL.
W 3	No carbonates.
W 4	1 ton calcite.
W 5	2 tons calcite.
W 9	2 tons dolomite.
W 10	2 tons magnesite.
W 13	6 tons calcite.
W 14	6 tons magnesite.



## RECENT STUDIES ON SCLEROTIUM ROLFSII SACC.

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### ECONOMIC IMPORTANCE OF THE FUNGUS

As a parasite *Sclerotium rolfsii* Sacc. is of the same economic importance to the South as *Sclerotinia libertiana* Fck. is to some of the more northern States. Rolfs (12, 13, 14)<sup>1</sup>, in Florida, and Earle (1), in Alabama, found it to be serious on tomatoes. In Louisiana, Edgerton and Moreland (3, p. 19) also found it upon tomatoes. Fulton (4, p. 3), states that it often ruins the pepper crop in Louisiana. Wolf (2, p. 142-146), in Alabama, and McClintock (10), in Virginia, have fully recognized its economic importance as the causal organism of a peanut trouble. Earle and Rogers (2) and Wolf (21) have studied a citrus disease due to *Sclerotium rolfsii*, and Peltier (11) has also found it on cultivated perennials. Godfrey (5) found it on wheat. In Texas, the writer observed *Sclerotium rolfsii* attacking cantaloupes, tomatoes, peppers, peanuts, watermelons, young cotton seedlings, sweet potatoes, radish, cabbage, young corn plants, Bermuda grass, and a large number of weeds. It is to be regretted that there are no definite statistical data on crop losses from the attacks of this fungus. However, a conservative estimate might place these losses at about 5 per cent of the southern crops. In Texas *Sclerotium rolfsii*, although widely distributed, seems restricted to the sandy or sandy loam soils, the greatest damage occurring in the wet seasons. The writer has repeatedly failed to find it on the heavy waxy soils where *Ozonium omnivorum* Sh. is so prevalent. The fungus is an air-loving organism, so it commonly finds an ideal environment in the light sandy soils.

*Sclerotium rolfsii*, although apparently occurring only in the South, has been recently reported by Peltier (11) for the first time in Illinois. It seems rather difficult to account for its sudden appearance there. Halsted (6,7), in working with a pure culture of this fungus originally derived from Florida, found considerable difficulty in obtaining positive infection with plants in New Jersey. The fungus has, so far as known, not been found there as a field trouble. The writer had difficulty in infecting healthy plants in Delaware, in 1912, with a pure culture sent to him from Alabama. It is to be remembered, too, that in Delaware *S. rolfsii* does not occur as a field parasite. It is probable that in Illinois the fungus was introduced with imported ornamentals from the South. In

<sup>1</sup> Reference is made by number (italic) to "Literature cited," p. 37-138.

that case, and as explained by Peltier (11), the extremely wet summer in 1915 at Urbana, Ill., together with an average low temperature of 74° F. may account for the sudden appearance of the disease. *S. rolfsii* may not be able to withstand the cold winters of Illinois, in which case there should be no fear of its further spread. However, should it withstand the cold winters of Illinois, it would be reasonable to suppose that it is adapted to colder climates, and that if not guarded against may spread to other Northern States. Failure by Halsted and by the writer to infect plants in New Jersey and in Delaware may be explained by the fact that both worked with strains grown too long on artificial media. This, however, needs further verification.

#### HOST PLANTS

*Sclerotium rolfsii* is known to attack a large number of various genera and species of plants. Table I shows the wide adaptability of this fungus to different hosts.

TABLE I.—*Hosts affected by Sclerotium rolfsii*

Host.	Authority.	Year.	State.
<i>Arachis hypogaea</i> .....	Earle, F. S. ....	1900.....	Alabama.
	Rolfs, P. H. ....	1890.....	Florida.
	McClintock, J. A. ....	1917.....	Virginia.
	Taubenhaus, J. J. ....	1917, 1918.....	Texas.
<i>Beta vulgaris</i> .....	Earle, F. S. ....	1900.....	Alabama.
	Rolfs, P. H. ....	1890.....	Florida.
<i>Brassica oleracea</i> .....	.....do.....	1890.....	Do.
	Taubenhaus, J. J. ....	1916, 1918.....	Texas.
<i>Capsicum annum</i> .....	Fulton, H. R. ....	1908.....	Louisiana.
	Taubenhaus, J. J. ....	1917, 1918.....	Texas.
<i>Cucurbita</i> spp. ....	Rolfs, P. H. ....	1890.....	Florida.
	Taubenhaus, J. J. ....	1918.....	Texas.
<i>Citrullus vulgaris</i> .....	Rolfs, P. H. ....	1890.....	Florida.
	Taubenhaus, J. J. ....	1916, 1917, 1918.....	Texas.
<i>Chrysanthemum</i> spp. ....	Rolfs, P. H. ....	1890.....	Florida.
<i>Campanula</i> spp. ....	Peltier, G. L. ....	1916.....	Illinois.
<i>Colocasia esculenta</i> .....	Harter, L. L. ....	1916.....	Do.
<i>Daphne</i> spp. ....	Rolfs, P. H. ....	1890.....	Florida.
<i>Dianthus plumarius</i> .....	Peltier, G. L. ....	1916.....	Illinois.
<i>Dracoccephalum argense</i> .....	do.....	1916.....	Do.
<i>Edgeworthia papyrifera</i> .....	Fulton, H. R. ....	1918.....	Louisiana.
<i>Epilobium angustifolium</i> .....	Rolfs, P. H. ....	1890.....	Florida.
<i>Erigeron</i> spp. ....	do.....	1890.....	Do.
<i>Expatorium ageratoides</i> .....	Peltier, G. L. ....	1916.....	Illinois.
<i>Ficus carica</i> .....	Rolfs, P. H. ....	1890.....	Florida.
<i>Fragaria</i> spp. ....	Earle, F. S. ....	1900.....	Alabama.
<i>Gossypium hirsutum</i> .....	Rolfs, P. H. ....	1890.....	Florida.
	Taubenhaus, J. J. ....	1917, 1918.....	Texas.
<i>Hydrangea pani</i> .....	Rolfs, P. H. ....	1890.....	Florida.
<i>Ipomoea purpurea</i> .....	do.....	1890.....	Do.
	Taubenhaus, J. J. ....	1917.....	Do.
<i>Lycopersicum esculentum</i> .....	Rolfs, P. H. ....	1893, 1898.....	Do.
	Earle, F. S. ....	1900.....	Alabama.
	Taubenhaus, J. J. ....	1916, 1917, 1918.....	Texas.
<i>Phaseolus vulgaris</i> .....	Earle, F. S. ....	1900.....	Alabama.
	Fulton, H. R. ....	1908.....	Louisiana.
	Rolfs, P. H. ....	1890.....	Florida.

TABLE I.—Hosts affected by *Sclerotium rolfsii*—Continued

Host.	Authority.	Year.	State.
<i>Penstemon</i> spp. ....	Peltier, G. L. ....	1916. ....	Illinois.
<i>Phlox subulata</i> .....	do. ....	1916. ....	Do.
<i>Rheum rhabonticum</i> .....	Rolfs, P. H. ....	1896. ....	Florida.
	Taubenhaus, J. J. ....	1918. ....	Texas.
<i>Solanum tuberosum</i> .....	Earle, F. S. ....	1900. ....	Alabama.
	Rolfs, P. H. ....	1896. ....	Florida.
	Taubenhaus, J. J. ....	1917. ....	Texas.
<i>Saccharum officinarum</i> .....	Fulton, H. R. ....	1908. ....	Louisiana.
	Wakker, J. H. ....	1897. ....	Java.
<i>Solanum melongena</i> .....	Rolfs, P. H. ....	1896. ....	Florida.
	Taubenhaus, J. J. ....	1918. ....	Texas.
<i>Vigna sinensis</i> .....	Earle, F. S. ....	1900. ....	Alabama.
	Rolfs, P. H. ....	1896. ....	Florida.
	Taubenhaus, J. J. ....	1917, 1918. ....	Texas.
<i>Viola odorata</i> .....	Rolfs, P. H. ....	1896. ....	Florida.
	Taubenhaus, J. J. ....	1918. ....	Texas.
<i>Xanthosoma sagittifolium</i> .....	Harter, L. L. ....	1916. ....	Do.
	do. ....	1916. ....	Do.

Though *Sclerotium rolfsii* attacks a large number of hosts, its virulence is more pronounced on tender plants and growth. As a storage-rot its economic importance can not be overlooked. Pumpkins, squashes, cabbage, and Irish and sweet potatoes are often seriously affected under storage conditions when the necessary ventilation is lacking.

#### NAME OF THE DISEASE

Plant pathologists are aware of the necessity of standardizing names of plant diseases. The disease here considered seems to have as yet no standard name. Rolfs (12) and Fulton (4) named it "blight," and Earle (1) and Edgerton (3 p. 19) called it "Sclerotium wilt." McClintock (10) terms it "wilt," and Wolf (20, p. 142-146), working on a peanut disease, named it "Sclerotial rot." Peltier (11) does not give it any particular name, but merely refers to it as a "disease" or "rot." Stevens and Hall (18, p. 259-262) and the writer (19, p. 305) have referred to it as "southern blight." However, this term is misleading, since the bacterial blight of tomatoes named by Halsted (6), in Mississippi, as southern blight is generally accepted now as being caused by *Bacillus solanacearum* E. F. Sm. Neither would the term "Sclerotium rot" or "wilt" be tenable, since there are several species of *Sclerotium* fungi known to produce disease in plants. The term "southern Sclerotium rot" is therefore proposed. This will suggest the nature of the causal organism and its southern origin.

#### PATHOGENICITY AND RACIAL STRAINS

Few of the writers on *Sclerotium rolfsii* have mentioned an attempt to carry out pure culture inoculations to prove the pathogenicity of the fungus. It seems to have been taken for granted that this fungus is a



parasite. Of those who report such attempts Wolf (20) may be mentioned. He inoculated various legume seeds by stirring in a small quantity of water containing a macerated vigorous culture of *S. rolfsii*. The inoculated seed was then planted in the greenhouse, in a soil which was previously treated with formaldehyde. As a result of this treatment positive infections were obtained with some of the legumes used. Positive infections with *S. rolfsii* on pepper plants were also recorded by Fulton (4, p. 3-8).

In order to establish definitely the pathogenicity of *Sclerotium rolfsii*, and also to determine whether or not there existed varietal or physiological strains in this organism, the inoculations reported in Table II were carried out. All hosts were covered with bell jars for 24 hours immediately after inoculation. All inoculations were performed in the greenhouse.

Although many more inoculations have been made than are reported in Table II, these will show that *Sclerotium rolfsii* is an active parasite.

A study of Table II shows further that there are no varietal or physiological strains in this organism. A strain isolated from tomatoes, for instance, will infect a large number of other hosts. This is also true when a strain from a cantaloupe is isolated. Table II also shows that no infection whatsoever could be obtained with a test tube culture 1 year old, even though the same was mixed with sterilized soil where it had no competition with any other of the soil floras and where it had plenty of available food. However, just as soon as a transfer was made from the 1-year-old culture it revived and assumed its normal virulence. It was then in no way different from a fresh strain of *Sclerotium rolfsii* recently isolated from a normally infected plant in the field.

*Sclerotium rolfsii* is truly parasitic, since it is not always necessary to produce infection through puncture inoculations. As shown in Table II, positive infections were obtained when a pure culture of the fungus was merely worked into the soil where moisture was present. These same observations were also reported by Fulton (4). However, Harter (8, 9) could not obtain any infection on *Colocasia esculenta* or *Xanthosoma sagittifolium* unless inoculations were made by means of a puncture. The writer has had similar experiences in his inoculations with the Irish and sweet potatoes (Pl. 5, B, C) as well as the orange and the apple. In these cases infections were possible only through a wound. On the other hand, however, as soon as infection took and the rot had progressed sufficiently all that was necessary then to infect a healthy tuber of Irish potatoes was to place the latter in close proximity to the former and infection would result without the aid of a puncture. In this case, apparently, the fungus assumed added vigor on the first inoculated tuber and was therefore capable of penetrating the other without the aid of a wound. In *Colocasia* and *Xanthosoma* the corms are protected by hard scales, and infection becomes possible only by means of a wound.

TABLE II.—Inoculation with pure cultures of *Sclerotium rolfsii* on various hosts

Source and age of culture.	Date of inoculation.	Host inoculated.	Method.	Result.	Controls.
1a. Delaware strain, 1 year old	July 9, 1917	15 tomato plants, 6 weeks old.	Fungus worked into sterile soils. <sup>b</sup>	All healthy.....	5, all healthy.
Do.....	do.....	10 peanut plants.	do.....	do.....	Do.
Do.....	do.....	5 cabbage heads.	Puncture.....	do.....	2, both healthy.
Do.....	do.....	10 tubers Irish potatoes.	do.....	do.....	5, all healthy.
1b. Transfer from 1a, 10 days old.	do.....	15 tomato plants, 6 weeks old.	Fungus worked into sterile soil.	12 positive infections.	Do.
Do.....	do.....	10 peanut plants.	do.....	6 positive infections.	Do.
Do.....	do.....	3 cabbage heads.	Puncture.....	All positive infections.	2, 1 healthy, 1 black-rotted.
Do.....	do.....	10 tubers Irish potatoes.	do.....	do.....	5, all healthy.
1c. Fresh strain isolated from cantaloupe, 10 days old.	July 15, 1917	6 tomato plants, 5 weeks old.	Fungus worked into soil.	5 positive infections.	Do.
Do.....	do.....	14 sweet-potato plants.	do.....	9 positive infections.	3, all healthy.
Do.....	do.....	2 watermelon fruits.	Puncture.....	Both positive infections.	2, both healthy.
Do.....	do.....	20 sweet-potato roots.	do.....	17 positive, 3 soft rotted from Rhizopus.	6, all healthy.
Do.....	do.....	10 cantaloupe fruits.	do.....	All positive infections.	2, both healthy.
Do.....	do.....	6 gourd fruits.	Puncture.....	do.....	Do.
1d. Fresh strain isolated from tomato in field, 8 days old.	July 16, 1917	8 tomato plants, 12 weeks old.	Fungus worked into soil.	5 positive infections.	Do.
Do.....	do.....	3 peanut plants, 9 weeks old.	do.....	All positive infections.	Do.
Do.....	do.....	10 sweet-potato plants.	do.....	9 positive infections.	5, all healthy.
Do.....	do.....	20 corn plants, 3 weeks old.	do.....	8 positive infections.	10, all healthy.
Do.....	do.....	6 watermelon fruits.	Puncture.....	All positive infections.	2, both healthy.
Do.....	do.....	5 cantaloupe fruits.	do.....	do.....	Do.
Do.....	do.....	10 unripe banana fruits.	do.....	All positive infections, anthracnose present.	5, 4 healthy, 1 rotted from anthracnose.
Do.....	do.....	6 sweet-potato roots.	do.....	5 positive infections, 1 soft rotted from Rhizopus.	3, all soft-rotted from Rhizopus.
Do.....	do.....	12 oranges.	do.....	All positive infections.	4, all healthy.
Do.....	do.....	12 apples, variety unknown.	do.....	do.....	3, all healthy.
Do.....	do.....	5 squashes.	do.....	do.....	2, both healthy.
Do.....	do.....	5 pepper plants, 7 weeks old.	Fungus worked into soil.	4 positive infections, 1 doubtful.	Do.
1e. Fresh strain isolated from peanut in field.	Aug. 19, 1917.	7 tomato plants, 5 weeks old.	do.....	5 positive infections.	Do.
Do.....	do.....	7 peanut plants, 5 weeks old.	do.....	All positive infections.	4, all healthy.
Do.....	do.....	12 sweet-potato plants, 4 weeks old.	do.....	do.....	5, all healthy.
Do.....	do.....	10 tubers Irish potatoes.	Puncture.....	8 positive infections.	4, all healthy.
Do.....	do.....	8 sweet-potato roots.	do.....	All positive infections.	5, all healthy.

<sup>a</sup> Culture originally obtained from Alabama.<sup>b</sup> Fungus mycelium crushed up in sterile water, then worked in about 25 inch deep.<sup>c</sup> Black-rotted by *Pseudomonas campestris* (Fum.) Bw. Sm.

## PERIOD OF INCUBATION

The findings of the writer substantiated those of others—namely, that with growing plants such as tomatoes, peanuts, corn, cotton seedlings, sweet potatoes, and others the period of incubation ranges from 2 to 4 days, depending on the tenderness of the growing tissue. Wilting usually begins after the second day, and the plant usually dies in from 4 to 6 days after inoculation. In inoculations of tubers of Irish potatoes, infection becomes apparent in about 6 days. After 15 days the potatoes are about half rotted (Pl. 5, B), the injury being a typical “melter,” the tubers becoming very soft and at the least pressure rupturing and liberating a clear liquid with an agreeable odor of fermentation. With sweet potatoes the period of incubation is about the same as that of Irish potatoes, with the difference that the rot produced is not of the “melter” type. The infected tissue becomes browned, water-soaked at first but firm, then hard and stringy (Pl. 5, C). With the cantaloupe the period of incubation is usually from 3 to 6 days, after which the rot progresses very rapidly. If the inoculated melon is so placed that the point of infection touches the glass, the rot works so rapidly that it practically melts away half of the fruit, leaving a ragged cavity (Pl. 3, E). On the other hand, if after inoculation the fruit is so placed that the point of infection is turned upward and not allowed to touch the glass, the rot progresses slowly without producing a rapid soft rot. At the same time the fungus hyphae permeate the fruit (Pl. 3, F) and form a luxuriant growth which spreads all over the surface of the cantaloupe (Pl. 3, D). After the contents of the fruit are destroyed by the fungus, all that remains is a compacted mass of mycelium, which later rounds itself up into one mass of small sclerotia (Pl. 3, C). With the watermelon or the squash the period of incubation varies from 8 to 10 days, the inoculated fruit dry-rotting very slowly. The incubation period for the banana varies from 4 to 8 days, and for the orange and the apple from 6 to 10 days. Once infection starts these fruits rot very rapidly.

## MODE OF INFECTION

It has already been pointed out that abrasion of the host is not necessary for infection. This is especially true with tender growing plants. Of paramount importance to infection may be considered moisture and especially air. For successful soil inoculations it is necessary to cover the fungus not more than  $\frac{1}{2}$  to 1 inch deep. Numerous laboratory experiments indicate that no infection is possible if either the mycelium or the sclerotia are buried more than 5 inches deep. This suggests that deep plowing would control the trouble. Furthermore, infection seems possible only where a young and actively growing culture is used.

Infection seems to be favored by an enzyme secreted by the advancing mycelial strands. Examination of the roots or tubers infected with

*Sclerotium rolfsii* always shows a distinct zone of demarkation preceding the rotted area. Careful microscopic examination of the tissue in this zone does not show the presence of any fungus hyphae. Numerous attempts in culturing such tissue failed to produce any growth whatsoever. Moreover, the fungus always advances in large tufts or strands, which are composed of numerous hyphae joined together for the purpose, apparently, of secreting more enzymes to kill the host cells and to facilitate the more rapid progress of the rotting. In the large amount of embedded material which was sectioned and stained, no evidence was found to indicate that the growing tips of the advancing hyphae penetrate the cells of the host. Their purpose is apparently enzymic secretion. On the other hand, penetration seems always to be effected by the secondary hyphal branches which are formed at some distance below the growing tips. These usually penetrate the host through the stomata of the epidermis, then work inward; or they may break directly through the epidermal cells.

With starchy roots, such as those of sweet and Irish potatoes, the fungus apparently has difficulty in penetrating the cells which are gorged with starch. Studies and observations in this direction show that the enzyme merely dissolves the middle lamella of the cells (fig. 1, II) and that the fungus hyphae are not found within the cells but only between them, where the middle lamella has disappeared. On the other hand, with soft tissue, especially with cantaloupes, the fungus hyphae are capable of piercing the cell walls and of working both within and between the cells (fig. 1, A, B, C). In migrating from one cell to another of the host tissue, the tip end of the mycelium attaches itself closely to the cell wall, then rounds up into a small ball, which develops a sharp point that pierces the cell wall. When this is accomplished the tip end again swells slightly, then straightens out, and grows in the usual way (fig. 1, B, C).

#### SYMPTOMS

The symptoms on actively growing plants are very striking. With the tomato, sweet potato, peanut, pepper, corn, as well as other tender plants, infection invariably starts at the foot of the plant from  $\frac{1}{2}$  to 1 inch below the ground level. Early infection is at first manifested by deep brown lesions. At this stage the host exhibits a slight wilting, as though suffering from a lack of water in the soil. Soon after, however, the lesions become covered with white radiating mycelium which encircles the foot of the plant. At this stage the epidermis and the cambium become water-soaked but remain firm, the foliage droops, loses its green color, and the plant never revives. The fungus seldom works to any considerable extent upward on the main stem; but it always works downward toward the main root and rootlets, especially those which are nearest to the surface. If a dead plant is pulled out, its roots and

rootlets are usually found covered with a white web of the mycelium of the causal fungus. If the soil is kept moist and the dead plant remains untouched, the fungus will grow out on the surface of the soil in radiating

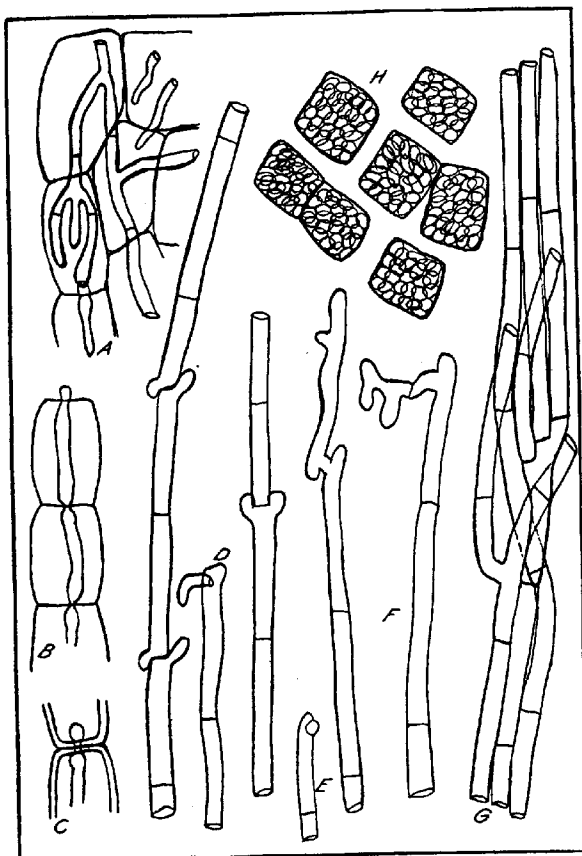


FIG. 1.—A, intracellular nature of *Sclerotium rolfsii* hyphae in cantaloupe tissue; B and C, manner in which the fungus pierces host cells; D, E, and F, method of budding and formation of new mycelial growth; G, manner of growth of mycelium, forming strands; H, dissolved middle lamellae of host cells.

fans around the foot of the dead plant (Pl. 6, A). With sweet potatoes in the seed bed the fungus often attacks young sprouts as soon as growth starts, in which case the mycelial strands work their way upward and

invade the entire tender stems (Pl. 5, A), which soften considerably and become covered with minute sclerotia. The fungus then works downward and rots the mother sweet potato. With stored cabbage the rot is confined to the two outermost layers of the head, which blacken and turn soft (Pl. 4, A).

#### MANNER OF GROWTH

The fungus was first studied by Halsted (6) and later by others, who, however, did not give it a specific name. Saccardo (15) named it *Sclerotium rolfsii* from specimens sent to him by Stevens (17, p. 660-661). In pure culture the growth of this organism is very distinct and can not easily be mistaken for any other species of *Sclerotium* fungus. Broadly speaking, this organism is little influenced by the kind of artificial medium on which it grows. Its mycelium is always white, fluffy, usually growing in strands and in radial fans (Pl. 4, B). This is especially true when an infected fruit is placed in a bell jar in contact with the glass. In a very short time the fungus grows out luxuriantly from the host on the surface of the glass, on which it forms beautiful radial fans (Pl. 6, B). The sclerotia, in pure cultures, are very little influenced as to size by the nature of the medium. In general they are of the size of a mustard seed (Pl. 4, B; 3, A). This, in fact, agrees with the general description of other workers. However, the size of the sclerotia is decidedly influenced by the kind of host which the causal organism infects. For instance, on cantaloupes and tomatoes the sclerotia are about the size of a mustard seed (Pl. 4, C). However, on the orange the sclerotia assume such large proportions (Pl. 3, B) as to resemble not those of *Sclerotium rolfsii* but rather those of *Sclerotinia libertiniana* Fck. However, the writer had no trouble to plant these large sclerotia on artificial media and obtained again the normal growth of *Sclerotium rolfsii*, with its accompanying mustard-seed-like sclerotia. On the apple no sclerotia were formed at all. No experiments have been made to determine the effect of fruit acids on the size of the sclerotia of *Sclerotium rolfsii*, although it seems probable that the acid in the orange is responsible for the abnormally large development of the sclerotia. Peltier (11) has similarly observed that the sclerotia of *Sclerotium rolfsii* on cultivated perennials in Illinois were much larger in size than those found by the other workers. Similar observations are recorded by Smith (16). This would seem to indicate that the kind of host has an influence on the size of the sclerotia of this fungus.

One further peculiarity which the writer observed in the growth of the mycelium of this fungus is worthy of mention. Growth, instead of proceeding indefinitely at the terminal end of the original mycelial thread, comes to a standstill, a bud is developed near the tip end of the terminal cell, and only the bud continues growth (fig. 1, D-F). The hyphae are seldom found growing singly but always appear in groups of several branches (fig. 1, G), which often anastomize and form regular strands.

## SEXUALITY

The observations of the writer lead him to believe in the existence of plus and minus strains in *Sclerotium rolfsii*, although they are only rarely met with. In June, 1917, an isolation of the casual fungus was made from damped-off cotton seedlings in the greenhouse. The infected tissue was first dipped for one second in a solution of 1 part corrosive sublimate in 2,000 parts of water, then carefully rinsed in sterilized water. It was then crushed with sterile forceps and mixed with melted and properly cooled medium, which was poured in a plate. After four days' growth in the Petri dish, sclerotia formed at the line where two colonies met (Pl. 4, D). This at once suggested a sexual act. Numerous transfers were made of the apparently different strains and were marked plus and minus. Whenever these strains were planted in the same Petri dish, sclerotia would always form at the line of union of colonies of the two strains. This was repeated many times with the same results. On the other hand, when each of these strains was planted separately few or no sclerotia would form. Moreover, when other varieties not supposed to have sexual strains were planted in the same Petri dish the sclerotia would form at random, and none would develop at the place of union of the two colonies (Pl. 3, A). Unfortunately, during a brief absence of the writer on emergency war work the plus and minus strains of *S. rolfsii*, together with many other cultures, were thrown out by a temporary employee in the laboratory.

## SUMMARY

(1) *Sclerotium rolfsii* is prevalent throughout the Southern States. It has also been found recently in Illinois.

(2) The fungus attacks a large variety of cultivated crops in the field, ornamentals included. It also causes a serious trouble in stored vegetable product.

(3) The fungus is a true parasite, found mostly in the light sandy loams. Air and moisture are both necessary for infection. If buried too deep in the soil the organism apparently dies, hence deep ploughing is suggested as a control measure.

(4) There are no varietal nor physiological strains in *Sclerotium rolfsii*.

(5) The period of incubations varies from two to six days.

(6) The size of the sclerotia in pure cultures is little influenced by the medium used. It is, however, considerably influenced by the host. On the orange the sclerotia assume unusual proportions, resembling more the sclerotia of *Sclerotinia libertiana*.

(7) The mycelium of *Sclerotium rolfsii* always appear in strands or in radial fans.

(8) The individual mycelial threads seem incapable of indefinite growth at the terminal cells of the hypha. New growth is effected by means of a bud developed at the terminal cell of the mycelium.

(9) There is a strong indication of plus and minus strains in *Sclerotium rolfsii*.

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PLATE 3

*Sclerotium rolfsii*:

A.—Two colonies in same plate, both apparently of the same sexual strain. No sclerotia formed at the point of union of the two colonies.

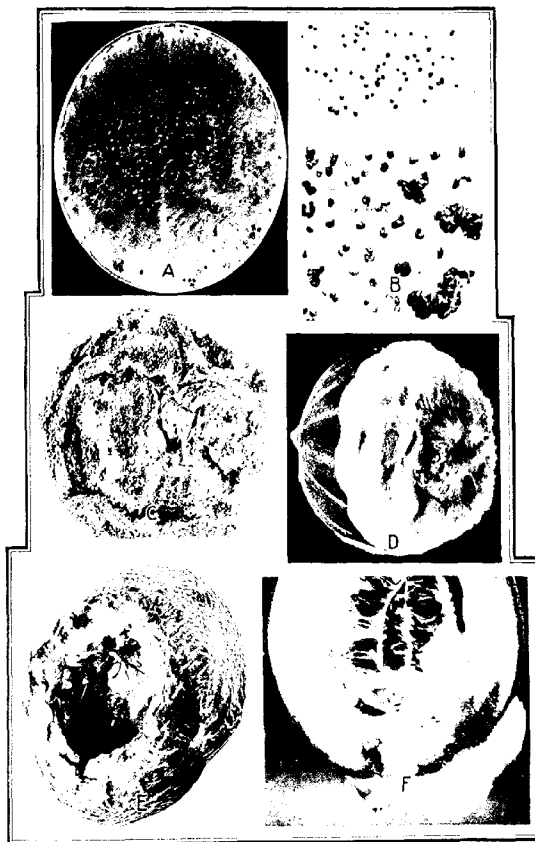
B.—Large sclerotia from inoculated orange.

C.—Late stage, showing cantaloupe reduced to a mass of mycelium and sclerotia.

D.—Inoculated cantaloupe fruit, the point of infection being free and away from the bell jar glass. Earlier stage than C.

E.—Infected cantaloupe, lying close to bell jar at point of infection. This shows the melting away of that part of the fruit in closest contact with the glass, leaving a ragged hole.

F.—Cross section of an infected cantaloupe, showing penetration of fungus into interior of fruit.



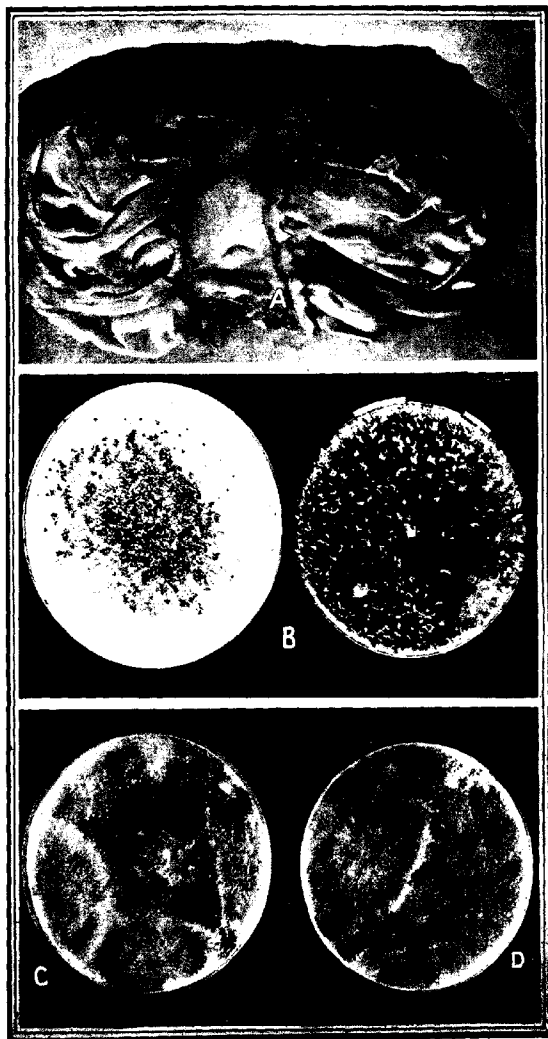


PLATE 4

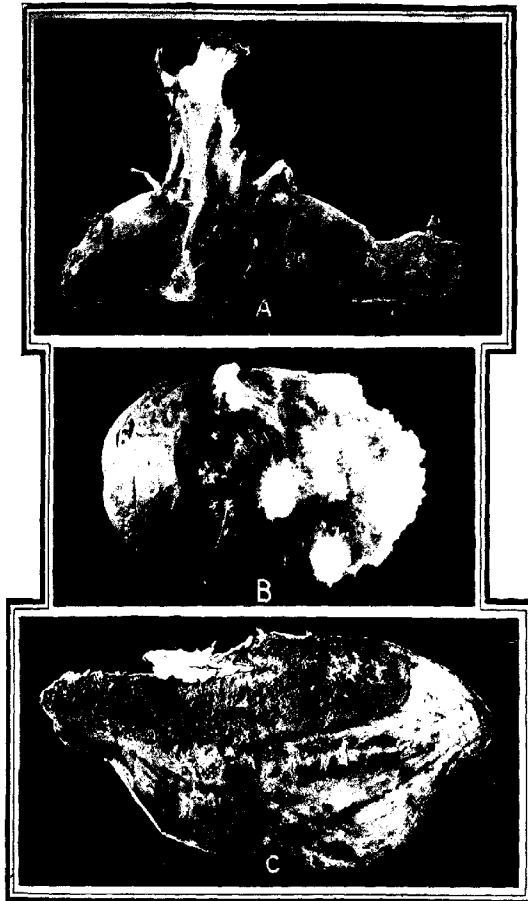
*Sclerotium rolfsii*:

- A.—Cabbage artificially inoculated. The rot is confined to the outer layers of the head.
- B.—Cultures on artificial media.
- C.—Mustard-seed-like sclerotia on cantaloupe.
- D.—Formation of sclerotia at point of union of apparently plus and minus strains.

PLATE 5

*Sclerotium rolfsii*:

- A.—Sweet potato in seed bed, showing method of natural infection of young sprouts.
- B.—Infected Irish potato, "melter" stage.
- C.—Longitudinal section of infected sweet potato, showing nature of rot.





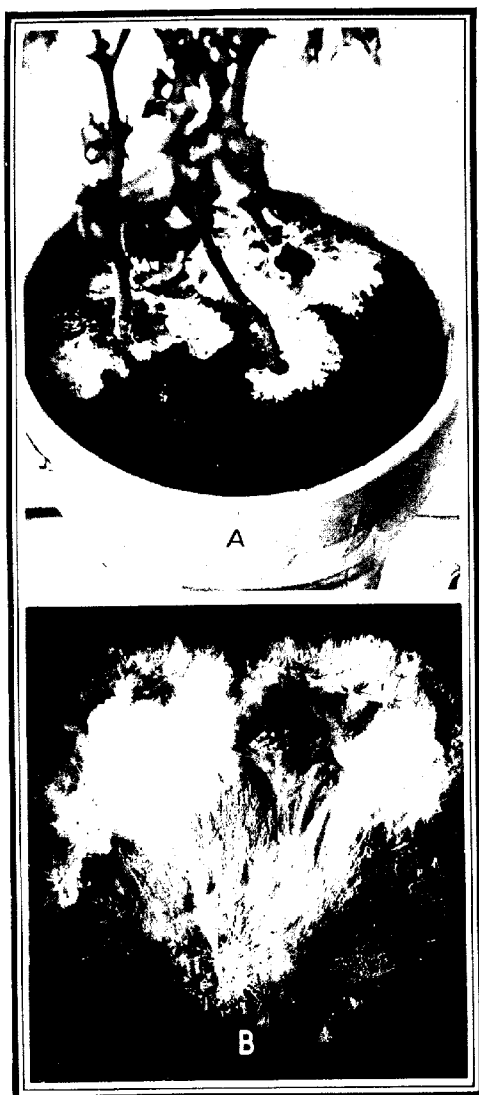


PLATE 6

*Sclerotium rolfsii*:

- A.—Dead tomato plants, showing fungus growing out on the surface of the soil in radial fans.
- B.—Growth on glass of bell jar, showing radial fans.



## EFFECT OF VARIATION IN MOISTURE CONTENT ON THE WATER-EXTRACTABLE MATTER OF SOILS

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The question of the possible effect produced on the water-soluble matter by variations in the moisture content of the soil became of interest in connection with investigations of the water extracts of soils carried on at this laboratory.<sup>1</sup> In the work referred to, the soils were maintained at all times as near their optimum moisture contents as was practicable with the large quantities (1,800 pounds) of soil involved. Some variation was, however, found to take place between waterings and is deemed to be inevitable in experiments of that type. The purpose of the present study was to determine to what extent variations in moisture content of soils modify the magnitudes of their water extracts and thus vitiate conclusions drawn from our own and similar experiments.

### DESCRIPTION OF SOILS

The two soils studied herein are regarded as typical of the two classes used in much of the recent work of this laboratory. No. 1 is a Yolo silty clay loam and is the same soil as that called "No. 1" in the investigations of Stewart.<sup>1</sup> No. 2 is a sandy loam very similar to the "No. 11" soil described in the same publication. The portions of the soils used in this investigation were taken from bins in which they have been stored for several years and hence show a relatively great accumulation of water-soluble matter. They were practically in the air-dry condition, No. 1 containing 5 per cent moisture and No. 2 about 2.5 per cent. The optimum water content for the silty clay loam soil is 22 per cent, while that of the sandy loam is 15 per cent.

### PROCEDURE

Four 500-gm. portions of soil No. 1 were placed in quart Mason jars and brought to a moisture content of 10, 15, 20, and 25 per cent, respectively, making 16 jars or samples in all. The same procedure was followed for soil No. 2, except that the moisture contents were 5, 10, 15, and 20 per cent. These moisture contents were chosen as those covering the range of possible moisture variations of these soils in the field during the season. The lowest moisture content maintained is approximately the air-dry condition, while the highest is slightly above optimum for each soil.

<sup>1</sup> STEWART, GUY R. EFFECT OF SEASON AND CROP GROWTH IN MODIFYING THE SOIL EXTRACT. *J. N. Jour. Agr. Research*, v. 12, no. 6, p. 311-308, 24 fig., pl. 14. 1918. Literature cited, p. 304-308.

The jars were then buried in the ground to the level of the soil inside. The covers were set on loosely to allow free circulation of air and at the same time prevent excessive evaporation. The area occupied was shaded to prevent heating the jars and metallic covers by the sun's rays. At frequent intervals—two weeks or oftener, depending on the weather—enough distilled water was added to bring the soil in each jar to its correct moisture content. Since this is a study of the effect of moisture content, it was necessary to keep that factor constant.

Two days after the soils were first moistened, one jar of each soil at each of the various moisture contents was removed and mixed. A 50-gm. portion was dried to constant weight to check the moisture percentage. Soil equivalent to 340 gm. in the water-free state was extracted with 1,700 cc. of distilled water, inclusive of the water in the soil, thus insuring a 1 to 5 extraction, which was made after the manner described by Stewart.<sup>1</sup> The remainder of the soil was used to determine the concentration of the soil solution, by the freezing-point lowering, as described by Bouyoucos and McCool.<sup>2</sup> This procedure was repeated three times during the period of the experiment at varying intervals of time; the total length of time between the first and last sets of analyses was 22 weeks, which is longer than a normal growing season.

Table 1 gives a complete résumé of the results obtained. The concentration of the soil solution is reported in atmospheres of osmotic pressure, the analyses of the water extracts as parts per million of the water-free soil.

#### DISCUSSION OF RESULTS

Knowledge of the inherent variations in the methods used is very essential in a study of this nature. For a detailed description of the methods employed reference is made to two recent publications from this laboratory.<sup>3</sup> It is desirable to draw conclusions as to differences only where such variations are of considerable magnitude, 20 per cent or more. It is also evident that comparisons should be made only between results obtained at the same sampling date, since it is known that there are seasonal fluctuations due to other factors.

OSMOTIC PRESSURE OF THE SOIL SOLUTION AND TOTAL SOLIDS.—These two determinations are placed together for consideration because of the fact that they are known to be directly related, as has been shown by Hoagland.<sup>4</sup> The most striking feature shown is the decrease of soluble matter in soil

<sup>1</sup> STEWART, GUY R. *OP. CIT.*

<sup>2</sup> BOUYOUCOS, G. J., and MCCOOL, M. M. THE FREEZING-POINT METHOD AS A NEW MEANS OF MEASURING THE CONCENTRATION OF THE SOIL SOLUTION DIRECTLY IN THE SOIL. *Mich. Agr. Exp. Sta. Tech. Bul.* 22, p. 527-531, 2 fig. 1915.

<sup>3</sup> STEWART, GUY R. *OP. CIT.*

CHRISTIE, A. W., and MARTIN, J. C. THE VOLUMETRIC DETERMINATION OF SULFATES IN WATER EXTRACTS OF SOILS. *J. Soil Science*, v. 4, no. 6, p. 477-479. 1917.

<sup>4</sup> HOAGLAND, D. R. THE FREEZING-POINT METHOD AS AN INDEX OF VARIATIONS IN THE SOIL SOLUTION DUE TO SEASON AND CROP GROWTH. *J. Agr. Research*, v. 12, no. 6, p. 369-395, 8 fig. 1918. Literature cited, p. 394-395.

No. 2, 20 per cent moisture. It might be stated here that this moisture content produced a water-logged condition, which will be discussed later. The percentage of decrease is noticeably greater at the later sampling dates than at the earlier, 20 per cent on May 13 and 60 per cent on October 28; and there is reason to believe they would continue to increase in divergence. Although that is the most striking feature, it is also noted that there is a general trend upward from the lowest moisture content to the optimum in both soils.

TABLE I.—Concentration of soil solution

SOIL NO. 1, SILTY CLAY LOAM

Moisture content.	Date of sampling.	Osmotic pressure.	Total solids.	Nitrate (NO <sub>3</sub> ).	Sulphate (SO <sub>4</sub> ).	Phosphate (PO <sub>4</sub> ).	Potassium (K).	Calcium (Ca).	Magnesium (Mg).
Per cent.		Atmos.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.
10. ....	May 13	1.07	1,208	244	194	3.8	54	90	51
	June 11	1.57	1,198	239	190	4.4	39	101	43
	July 22	1.35	1,422	312	186	2.9	18	99	44
	Oct. 28	1.35	1,345	274	201	3.7	41	112	69
15. ....	May 13	1.90	1,202	240	212	3.3	58	90	51
	June 11	1.44	1,287	257	212	3.0	38	102	45
	July 22	1.95	1,547	285	213	2.8	40	105	54
	Oct. 28	1.79	1,476	291	219	4.0	57	113	85
20. ....	May 13	1.55	1,302	244	225	3.7	62	92	47
	June 11	1.66	1,317	359	225	3.2	45	67	46
	July 22	1.66	1,551	327	225	3.2	40	100	53
	Oct. 28	1.72	1,482	259	244	2.6	52	122	61
25. ....	May 13	1.32	1,300	220	228	3.5	57	90	45
	June 11	1.70	1,345	245	226	3.0	40	102	47
	July 22	1.58	1,506	237	217	2.3	45	99	45
	Oct. 28	2.11	1,502	252	240	4.2	54	121	65

SOIL NO. 2, SANDY LOAM

Moisture content.	Date of sampling.	Osmotic pressure.	Total solids.	Nitrate (NO <sub>3</sub> ).	Sulphate (SO <sub>4</sub> ).	Phosphate (PO <sub>4</sub> ).	Potassium (K).	Calcium (Ca).	Magnesium (Mg).
Per cent.		Atmos.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.	P. p. m.
5. ....	May 13	0.42	494	131	51	4.7	37	47	17
	June 11	.74	345	174	46	4.0	28	57	13
	July 22	.59	715	131	50	4.5	19	62	17
	Oct. 28	1.01	613	159	52	7.4	38	80	26
10. ....	May 13	.56	504	128	54	4.0	43	50	17
	June 11	.79	361	133	49	3.2	31	53	13
	July 22	.55	718	102	54	4.1	35	70	19
	Oct. 28	1.04	685	190	56	9.5	45	85	15
15. ....	May 13	.54	541	135	54	4.3	40	50	18
	June 11	.85	379	133	49	3.7	33	60	13
	July 22	.72	700	116	41	4.6	37	71	19
	Oct. 28	1.02	773	210	60	5.0	43	90	18
20. ....	May 13	.21	415	64	53	6.1	42	47	15
	June 11	.69	321	Trace.	50	3.5	26	37	7.5
	July 22	.66	375	Trace.	48	5.3	28	40	16
	Oct. 28	.66	307	4	15	5.0	34	59	12

NITRATES.—The most notable thing is the depression in soil No. 2, 20 per cent moisture. This solute is the most affected by the excess water, as might be expected, anaerobic conditions having been produced. Another feature is the fact that at the optimum moisture content there is found the greatest quantity of nitrates, especially emphasized in soil No. 2 at the two later sampling dates.

SULPHATES AND PHOSPHATES.—The excess water does not have a depressing effect on these two solutes. The only evidence of any difference in quantity is the general trend upward in the water-soluble sulphate, coincident with the increased moisture in soil No. 1, which is relatively high in sulphate.

POTASSIUM.—There is a trend upward in quantity of this solute in soil No. 1 from the lowest to the highest moisture content; in soil No. 2 no significant effect is produced in the first two sets of determinations; however in the two latter it is seen that there are increasing quantities of potassium from soils of 5 per cent to 15 per cent moisture, and a decrease in the soil of 20 per cent moisture.

CALCIUM AND MAGNESIUM.—The striking feature is the depressing action of the excess water in soil No. 2 and the almost identical percentages of depression for the two solutes at the corresponding sampling date.

#### GENERAL DISCUSSION

In preparing the water extracts it was observed that the extract of the soil at the lowest moisture content filtered much faster than that at the highest, with a regular gradation between the two extremes, and that the difference in filtering speed was greater between the silty clay loams of highest and lowest moisture content than between corresponding samples of the sandy loam. A reasonable explanation may be found in the physical structure of the soil at the different moisture contents. It has been pointed out by Fippin<sup>1</sup> that the continued wet condition or dry condition does not produce any change in structure, but that alternate wetting and drying produce granulation, caused by expansion and contraction, which are directly proportional to the degree of wetting or drying. Thus the dryer silty clay loam may approach in structure that of a sandy loam. Klein<sup>2</sup> suggests that the increased granulation and hence greater access of water to the soil particle by drying may be overcome by the greater amounts of material held soluble in the soil at the higher moisture contents.

When a condition of saturation is reached there is a marked depression, as has been noted. The sandy loam soil at 20 per cent moisture was saturated, and water was standing on its surface a quarter of an inch in depth. This in itself is an indication of the results which might have been expected, and which were in fact obtained. This soil contained 5 per cent more water than its optimum, while soil No. 1 with 25 per cent moisture was 3 per cent above its optimum content and showed no apparent depression of solutes. Silty clay loam soils have a greater range between their optimum moisture contents and their saturation points than sandy loam soils because of the difference in soil texture.

The range of variation in moisture content covered in the present study was very much greater than the variations occurring in the investigations<sup>3</sup> referred to in the early part of this paper. In a season's work with these two soils it was observed that for soil No. 1 the moisture con-

<sup>1</sup> FIPPIN, Elmer O. SOME CAUSES OF SOIL GRANULATION. *In Proc. Amer. Soc. Agron.*, v. 2, 1915, p. 106-121, fig. 11-18. 1917.

<sup>2</sup> KLEIN, Millard A. STUDIES IN THE DRYING OF SOILS. *In Jour. Amer. Soc. Agron.*, v. 7, no. 2, p. 49-77, fig. Bibliography, p. 75-77. 1915.

<sup>3</sup> STEWART, Gay R. *OP. CIT.*

tem variation was between 22 per cent and 16 per cent, with an average of 19 per cent; for soil No. 2, the extremes of moisture variation were 16 per cent and 11 per cent, with an average of 13 per cent. These are variations of approximately 5 per cent from the optimum moisture contents and show the tendency of the soil to be slightly below the optimum in moisture. In the present study the only significant variations from the maximum quantity of extractable material are observed when the soils are either approaching the air-dry condition or are in a moisture-saturated condition. Looking again at the results recorded in the table and calling especial attention to those of soil No. 1 at 20 per cent moisture, at 15 per cent, and even at 25 per cent, and also to those of soil No. 2 at 15 per cent and at 10 per cent—these being variations of from 5 to 7 per cent below their optimum moisture contents—it is readily seen that at any one sampling date the quantities of water-extractable materials are exceedingly uniform and represent no significant differences. Therefore it is concluded that in water-extraction studies of soils the moisture content at which the soils are maintained need not necessarily be limited to a narrow range.

#### SUMMARY

- (1) The water-soluble constituents of two soils of very different type have been studied at four moisture contents.
- (2) The moisture contents approaching the air-dry condition show a decided tendency to depress the nitrates and potassium in both soils and the sulphates in the silty clay loam only. These depressions are reflected in the total dissolved material.
- (3) The excess water in the sandy loam soil causes a disappearance of nitrates and also decidedly depresses the potassium, calcium, and magnesium, these losses also being reflected in the total solids extracted.
- (4) Considerable variations in moisture contents of soils, provided the saturation point is not reached, do not appreciably modify the results obtained by the water-extraction method.





## PATHOLOGY OF DOURINE WITH SPECIAL REFERENCE TO THE MICROSCOPIC CHANGES IN NERVE TISSUES AND OTHER STRUCTURES

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Within the last five years on an average of from 45,000 to 50,000 complement-fixation tests have been made annually by the Bureau of Animal Industry for the diagnosis of dourine. The samples have been forwarded from Montana, North Dakota, South Dakota, Nebraska, Wyoming, Arizona, and New Mexico, especially from the Indian reservations in those States. Many improvements have been made in perfecting the technic of the complement-fixation test, thus affording better means of diagnosing dourine, which is the most essential factor in eradicating the disease. Diagnosis is the chief aim; but while all energies are directed toward that end other phases of dourine should not be overlooked, since they may help to explain directly or indirectly the variations in existing symptoms and the changes produced in the course of the disease. A careful perusal of the literature on this disease reveals the fact that in most articles on dourine the clinical picture—etiology, symptoms, and treatment—receives more attention than the pathological phase, the microscopic changes. These changes are often disposed of in a few sentences or short paragraphs, quoted usually from the older European descriptions of the microscopic findings. Very little consideration and study has been given in this country to determine whether microscopic changes as described in European cases of dourine are identical or different.

The object of this paper is to describe the microscopic lesions found in nerve tissues and other affected parts. The materials studied was taken from several well-developed chronic cases of dourine in horses from Montana and Iowa in which the disease had been recognized clinically and the animals had reacted to the complement-fixation test. The animals were shipped to the Bethesda, Md., experiment station of this bureau and kept under observation for nearly two years until they died. On post-mortem examination these animals showed lesions of dourine. An attempt will be made in this preliminary paper to correlate the clinical symptoms with the microscopic findings. In fact, the clinical symptoms afford chief guidance in selecting the tissues in which the structural changes were studied.

One of the difficulties confronting workers with nerve tissues is that in a short time post-mortem changes set in, and the beginning dissolution of nerve tissues may appear. Only the experienced technician accustomed to handling nerve tissues can apprehend the consequences. It is for this reason that the details of neurological technic will be described to enable the reader to follow the various steps necessary in bringing out all the details of the finer cytological changes which may occur in the tissue as the result of disease.

#### IMPORTANCE OF PROPER FIXATION

Before proceeding to make a post-mortem examination, proper fixing solutions must be ready to avoid all danger of disintegration. Fixation is a process by which the tissue is quickly killed and its structure rendered permanent.

The fixing agent must have the power to penetrate quickly before post-mortem changes have begun, as well as to give permanent results without distorting the shape, size, and position of the tissue elements. A fixing fluid of alkaline reaction should be avoided, since it tends to dissolve certain structural constituents rather than to fix. Some reagents, such as alcohol, while fixing rapidly cause a violent shrinkage of the tissue by inducing an unbalanced exosmosis of the fluid cell contents. In this way they bring about the shrinkage or collapse of the cells, which is decidedly objectionable.

The aim therefore should be to select fixatives in which the ingredients are mixed in such proportions that the swelling tendency of one will counteract the shrinkage tendency of the other. In removing nerve tissue, care must be taken that the portions exposed do not become dry and that the parts removed are not crushed or stretched. No single fixing fluid and no single staining process suffices to bring out finer structural changes. The neuroplasm of the ganglion cells is different from the neuroplasm of the nerve fibers not only in its appearance, composition, and behavior toward different fixatives but also in its affinity for different stains. Chromatophil granules are present in the ganglion cells but not in the nerve fibers. Myelin is present in the nerve fibers, but not in the nerve cells. Held has pointed out that chromatophil granules are brought out by the treatment of nerve tissue with alcohol or certain other fixing fluids. These appear according to their treatment as fine or coarse objects but are not visible in fresh nerve cells. The finer cytologic changes which are at the bottom of nervous diseases can be demonstrated only by modern methods of staining.

The structural distinction of nerve cells and nerve fibers is made as a matter of convenience for classification, description, and anatomical correlation. In reality the nerve cell and the nerve fibers constitute collectively the unit of the nervous system. This unit, called the neuron, is

composed of a cell body, nucleolus, nucleus, chromatic substance, achromatic substance, pigment, and processes. It would also include the protoplasmic processes with their gemmules and the axis-cylinder process, its collaterals, and at times a final arborization. The term chromatic or chromatophil substance, or Nissel's bodies, is applied to that portion of the cell substance which stains and has an affinity for methylene blue. It presents itself in many forms as irregular particles, smooth or dented fibers, or dumb-bell-shaped masses. The function of the chromatic substance has not up to the present time been definitely determined. The achromatic substance constitutes the greater part of the cell body and is made up of fine fibrils which pass through the cell with numerous anastomoses which gives this substance a finely reticular appearance. Certain observers consider the fibrils as the continuations of axones on their way into the processes. According to this view the achromatic substance is the all-important part of the cell.

The nerve fibers, on the other hand, are not independent elements but are those processes of the neuron known as the axon or axis-cylinder processes, which after their exit from the cell body extend and become invested in a protecting sheath. They are then known as the medullated nerves. The essential part of the nerve fiber is the central cord or axis cylinder, which is the only part concerned in transmitting the nerve impulse. The axis cylinder is composed of most delicate axis fibrillae embedded within a semifluid interfibrillar substance and is surrounded by a delicate sheath, the axilemma. The axis cylinder is surrounded on the periphery by a relatively thicker coat, the medullary sheath or white substance of Schwann, outside of which lies the delicate enveloping coat, the neurilemma. The medullary sheath is composed of a delicate reticulum of neurokeratin, the meshes of which are filled with a fatty substance, the myelin. This constitutes the majority of the peripheral medullated nerve fibers, but the medullated fibers of the spinal cord have no neurilemma.

The nerve fibers in the sympathetic system have no medullary sheath and are spoken of as the nonmedullated or gray fibers. The nerve cells and nerve fibers within the central nervous system are held together by a special supporting tissue, the neuroglia, which consists of an interlacing network of extremely fine fibrillae, the glia fibers, and glia cells. The cells are irregularly scattered in the course of the glia fibers.

Of the different fixing fluids, a 4 per cent solution of formaldehyde was used as a preliminary fixative more often than any other on account of its great power of penetration and rapid fixation. It must be followed, however, by other reagents such as Müller's fluid or Zenker's fluid, containing chrome salts, which bring out more clearly the ganglion cells, neuroglia, and axis cylinder. The chrome salt probably enters into chemical combination with the myelin, thus making possible the use of differential myelin sheaths stain.

Besides the general nuclear stains for cell protoplasm, selective stains were used, as the Van Gieson's stain, Nissel's stain, Pal's modification of Weigert's myelin stain, and Marchi's method of staining fatty degeneration in the myelin sheaths.

#### MANIFESTATIONS OF DOURINE

In reviewing the etiology of dourine we find that the unicellular protozoan *Trypanosoma equiperdum* is considered the cause of dourine. It is apparent that the disease, being transmitted in the act of coition, should show the principal lesions in the genital organs. But in all chronic cases of dourine, besides the lesions in the sexual organs pronounced derangements of peripheral nerves and the central nervous system are present, manifested by paralysis of nerves and atrophy of various groups of muscles. As trypanosomes can be found neither in the central nervous system nor in the peripheral nerves it must be assumed that the trypanosomes elaborate poisonous products or toxins which are responsible for the lesions. The presence or absence of lesions in acute cases will not be discussed, since all the observations were confined to chronic cases, nor will the symptoms and post-mortem examination be described in this paper.

The following tissues were selected: Brain, spinal cord, spinal ganglia, and peripheral nerves. No detailed study of muscles, skin, and genital organs was undertaken, though some preparations of these structures were made.

#### BRAIN

A number of pieces were taken from various parts of the brain, principally in the region of the lateral fissure (Sylvius), suprasylvian fissure, perisylvian fissure, and the sulcus rhinalis. Some of the sections were stained by the Van Gieson method, others with the Erythrosin-tuolin blue method. Both methods are used to study the general morphology of nerve structures, particularly the cell bodies of neurones. The nuclei appear bluish red, the ganglion cells and their protoplasmic processes red, the axis cylinder brownish red, the myelin sheaths yellow, the neuroglia fibers orange red, and the connective fibers deep red.

The sections from the different parts did not reveal any appreciable abnormality either in the nerve cells and their pericellular lymph spaces or in the blood vessels and their perivascular spaces. The cell and the nucleus took the stain uniformly; their size and external contour were unaltered. The blood vessels also appeared normal. Staining with the Nissel method showed no change in the chromatophil granules; neither was their amount or size increased or diminished. There was no change in regard to the staining ability or grouping of the nucleus or any indication of chromatolysis. Staining with Pal's modification of the Weigert method showed good contrast of the gray and white substance but no

evidence of degeneration. Staining with the Marchi method showed very light-brownish or yellowish coloration of the myelin and no black deposits. This differential action of osmic acid results from the fact that chromic acid and its salts deprive the normal myelin sheaths of their power of reducing osmic acid, while the chemically changed degenerating or abnormal sheaths retain this power. The method therefore gave positive black images of fibers in a state of degeneration, while the normal fibers remained unstained or only with light-colored yellow due to the bichromate in the Müller's fluid.

#### SPINAL CORD

The spinal cord contributed the bulk of material for the study of nerve changes. A number of pieces were taken from the cervical, thoracic, lumbar, and sacral regions. These pieces were fixed by methods best adapted for each particular stain. Fewer pieces were taken from the dorsal region than from the cervical or lumbar, as was indicated by the clinical symptoms and borne out by the microscopic examination. The sections from the anterior and middle dorsal region when stained with the Van Gieson method showed good contrast between gray and white substances, unaltered motor-ganglion cells, and no increase or reduction in neuroglia tissue or in the size of the medullated nerve fibers which constitute the dorsal, lateral, and ventral columns. The use of Nissel's method showed the chromatophil granules well stained, unaltered in amount, and with no indication of any degenerative changes. Pal's modification of the Weigert and the Marchi methods failed to show any evidence of alteration in the myelin in the medullated nerve fibers. The blood vessels appeared normal. Sections from the cervical and posterior dorsal regions will be discussed at the same time, since they showed similar lesions.

In sections from these regions stained with the Van Gieson method no appreciable changes could be observed either in the multipolar nerve cells or in the medullated nerve fibers of the gray or white substance. The Nissel method showed the chromatophil granules fairly well stained, slightly disarranged, but not enough to produce a significant deviation. Pal's modification of the Weigert method did not show sufficient difference in the medullated fibers to attach much importance to the lack of contrast between the gray and white substances. In sections stained by the Marchi method some of the medullated fibers in the dorsal horns showed a few scattered black clumps, especially in the extramedullary fibers outside the gray substance near the dorso-lateral groove. This was the first indication of degeneration of the myelin. It could not be detected by the other methods of staining. The medullated fibers of the ventral horns showed no black clumps. There were no black clumps in the medullated fibers of the dorsal columns in the white substance

In sections from the lower lumbar and sacral region stained by Van Gieson's method the deviations were more apparent than in the lumbar region.

The dorsal and ventral horns and the gray commissure appeared to have an increased quantity of neuroglia fibers. The Rolandic substance capping the dorsal horns was more distinct. The central canal in the middle of the gray commissure was enlarged. The single row of ependyma cells lining the canal were somewhat flattened, probably by pressure of spinal fluid, which distended the central canal. The central gelatinous substance, which is a modified neuroglia surrounding the central canal, appeared to be increased in amount and to contain hypertrophied glia cells. Simple dilatation of the central canal of the spinal cord is known as hydromyelia. When the dilatation becomes very extensive it is difficult to distinguish this condition from the hollowing out of the central canal by a process of softening known as syringomyelia, which is however usually found in the cervical region. Neither the motor-nerve cells of the ventral horns, the sensory-nerve cells within the dorsal horns, nor the cells of the column of Clark showed marked abnormality when stained by the Van Gieson method. There was, however, an increase of neuroglia tissue in the vicinity of the dorsal median groove, dorso-lateral grooves, and the entrance and course of the dorsal nerve roots. This increase of neuroglia was not sufficient to constitute sclerosis of the dorsal column. Sections that were stained by the Nissel method showed what may be regarded as the beginning of chromatolysis. The constituents that are most susceptible to influences are the Nissel bodies or chromatophil granules. While the mechanism of chromatolysis is still obscure, it is generally believed that the process represents the reaction of the cells to the disturbing forces, resulting in disintegration of the chromatophil granules in various parts of the cell, and is spoken of as peripheral, perinuclear, and disseminated.

Slight peripheral and perinuclear disintegration was observed in the sensory-nerve cells and to a less degree in the motor-nerve cells and the cells from the column of Clark. Neurologists generally consider that this latter condition is repairable or that the functional activity of the nerve cells is only partly impaired; but when the cell becomes deprived of its functional activity a further step in chromatolysis is produced that is not repairable, constituting a later stage of degeneration known as acromatolysis or plasmolysis. This latter condition was observed in sections from the lumbar region. No other method was so sensitive as the Nissel method in showing the earliest change of chromatolysis in the ganglion cells. Sections stained with Pal's modification of Weigart's method showed slight alterations. The bluish slate color of the medullated nerve fibers of the white substance showed good contrast with the yellowish stained gray substance which has only a limited number of medullated

fibers. The contrast was better seen in the ventral and lateral columns and was less apparent in the dorsal column where the medullated fibers appeared to be of a faded slate color approaching a yellowish tint, thus bordering on a beginning stage of degeneration. The change was sufficient to indicate a degeneration.

The Marchi method and Robertson's modification of Heller's method, both containing osmic acid, showed decided degeneration of the myelin in the medullary sheath of the nerve fibers. The intermedullary fibers within the gray substance contained a fair number of black clumps in the dorsal portion of their course. The extramedullary sensory fibers constituting the dorsal root showed more black clumps at the point of their entrance, the dorso-lateral groove. The black clumps gradually became fewer as the fibers entered the dorsal horns. The intramedullary fibers of the ventral horns, as well as their extramedullary portion, the motor-nerve roots, had only occasionally some black clumps. The distribution of these clumps in the medullated fibers of the white substance deserves special attention on account of the columns that are involved and the deduction of symptoms that could be made from a clinical standpoint.

The principal manifestations were found in the outer portion of the dorsal column, which is known as "funiculus cuneatus" or "Burdach's column." The black clumps were more numerous in the medullated fibers forming the outer boundary of the column or the fibers close to the dorsal-nerve roots and the dorsal horns. Fewer black clumps were present in the fibers near the inner boundary. The inner portion of the dorsal column is known as "funiculus Gracilis" or "Goll's column." Fewer black clumps were present than in the outer Burdach's column. Their number diminished as the fibers approached the dorsal medium septum. The medullated fibers of lateral and ventral columns showed no black clumps, except in the vicinity of the tips of the ventral horns. The changes observed in sections from the lower lumbar region were similar to the changes present in the sacral region. The degree of degeneration was a little more marked in the sacral region than in the lower lumbar region. The staining with the Marchi method and Robertson's modification of Heller's method showed the degeneration of the myelin in the medullary sheaths more pronounced and especially in the column of Burdach and to a less degree in the column of Goll, where the black clumps decreased in number. The black clumps gradually became fewer and disappeared entirely toward the distal end of the sacral region.

In man the subdivision of the white matter into various tracts with defined conducting pathways by which nerve impulse is conveyed has been worked out by research based on combined evidence of anatomical, pathological, and embryological investigation; but our knowledge of



these tracts in domestic animals is quite limited. We must recognize, however, in the white matter three classes of nerve fibers: those entering the cord from the periphery of the body, those entering from the brain, and those arising from the nerve cells situated within the cord itself. Fibers constituting the pathways for the transmission of impulses from lower to higher levels form the ascending tracts, while those fibers in which the impulse is conducted in the opposite direction form the descending tracts. The medullated fibers in the column of Burdach and the column of Goll were more affected than the fibers in other columns. The fibers of the dorsal column consist of two sets of axones. The afferent or sensory axones, which come from the cells of the spinal ganglia, enter as the dorsal roots of the spinal nerves and divide into two branches. The anterior ascending branches from the sensory pathway to the brain, extend to the fasciculus cuneatus and fasciculus gracilis, or corresponding tracts, to the nuclei in the medulla oblongata. The posterior descending branches extend backward for varying distances and give off numerous collaterals to the cells of the gray column, thus forming part of the mechanism for the mediation of reflex action. Some collaterals cross in the white commissure to the opposite side. The second set of axones arise from the smaller cells of the gray column. They enter the white matter and divide into anterior and posterior branches, forming the fasciculi proprii or ground bundles of the cord. The function of this set of axones is chiefly to associate various levels of the cord.

#### SPINAL GANGLIA

In removing the spinal cord, the spinal ganglia of the cervical and dorsal regions became detached. The ganglia of the lumbar enlargement and the sacral region alone were saved so that only a limited number of these ganglia could be examined. The same stains were used as for the staining of the brain and spinal cord. All ganglia appeared to be enlarged. The capsules were thickened. Many of the peripherally disposed nerve cells appeared normal in size. The nuclei and the chromatophil granules were well stained in some and much paler in others where the chromatophil granules were not only reduced in size but almost disintegrated and scattered among these average-sized cells. Smaller, irregular, shrunken cells were present, in which the disintegration was more marked. These latter cells took the staining very poorly and had the nucleus displaced toward the periphery. But even in these cells the nuclei had not entirely lost the staining property. The partial disintegration of the chromatophil granules and the pale color of the shrunken or sclerotic cells indicated varying degrees of chromatolysis. The interstitial tissues in the interior of the ganglia were increased in amount and showed in places clusters or groups of round-cell accumulations. In the sacral ganglia a greater number of shrunken cells were

present and chromatolysis had reached a stage where the chromatophil granules had disintegrated and in some of the cells totally disappeared. Here the name of plasmolysis is more applicable.

#### EXTRASPINAL NERVE TRUNKS

The great sciatic nerve was the only nerve trunk examined. The nerves from both sides were taken in each case, and transverse and longitudinal sections were made. The same stains were used as for the brain and cord, except that the Nissel method was omitted. The transverse sections were more instructive than the longitudinal. A number of the medullated fibers showed degeneration. A cross section of the fiber appeared as a circle with a dot in the center, corresponding to the axis cylinder. In many of the fibers the degeneration was so complete that the myelin as well as the axis cylinder disintegrated completely, leaving a granular mass behind. The circular outline of the cut fiber could not be distinguished. The funiculus therefore contained fewer circles which were separated by disintegrated material. The endoneurium was slightly increased in amount. The perineurium was more increased and the outlines of the individual funiculi became more distinct. There was also an increase in the number of connective tissue cells. The epineurium was more hypertrophied than the perineurium or the endoneurium. Scattered between the funiculi a number of perivascular inflammatory foci were present, besides the increase of irregularly scattered connective tissue cells. The left sciatic showed a greater number of degenerated fibers than the right. The Marchi method showed a fair number of black clumps in the interior of the funiculi. In longitudinal sections the black clumps were arranged in continuous rows in the more peripherally disposed funiculi. In the left nerve the rows of black clumps were longer, and more fibers were affected than in the right nerve.

#### SUMMARY

The microscopic examination of the brain showed no appreciable changes in the nerve cells, the supporting tissue, or in the blood vessels. In the cervical, anterior, and middle dorsal portions of the spinal cord lesions could not be demonstrated even with the most sensitive methods of staining; and in the posterior dorsal portion the lesions were very slight, gradually increasing in the lumbar enlargement and becoming most marked in the sacral region. Degeneration in the sensory ganglion cells was present in all stages, varying from the beginning stage of chromatolysis that could barely be detected by the Nissel method alone to advanced degeneration and disintegration of plasmolysis that was brought out by less sensitive methods. The motor ganglion cells and the cells in the column of Clark showed such slight alteration that it was difficult to trace chromatolysis in them. The nerve cells of the spinal

ganglia showed chromatolysis in varying degrees. The most marked changes were found in the sensory cells in the sacral region where disintegration of the chromatophil granules was followed by atrophy and sclerosis and was invariably accompanied by peripheral displacement of the nuclei. This was not observed in the nerve cells of the cord.

The degeneration of the myelin in the medullated fibers was even more pronounced than the degeneration in nerve cells. The black clumps of degenerated myelin stained by the osmic acid of the Marchi method were the characteristic feature of the endoneural and extraneural fibers in the gray substance in the dorsal horns and the dorsal nerve roots as well as of the fibers of the columns of Burdach and Goll in the white substance of the cord. The changes were limited to the lumbar and sacral region. In the sciatic nerve the degeneration was even more marked. We can therefore assume that the disturbances are of peripheral rather than central origin.

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## YELLOW-BERRY IN HARD WINTER WHEAT

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In an earlier publication<sup>1</sup> of the Kansas Agricultural Experiment Station, data were presented to show that yellow-berry in wheat is heritable and that improvement in the ability of wheat to resist the disease can be accomplished by breeding. The investigations reported therein have been extended, and some studies pertaining to the physiological processes that result in yellow-berry have been made. It is the purpose of this paper to present the results of these later studies.<sup>2</sup>

### THE NATURE OF YELLOW-BERRY

The yellow-berry problem has two aspects, the one practical, the other theoretical. The presence of yellow-berry in wheat causes it to grade lower and sell at a lower price than clear, hard wheat high in gluten. Furthermore, as Bailey says (1, p. 18):

If the kernels are soft in texture, or represent what is termed the "yellow-berry" condition, the percentage of flour will be reduced, since it is mechanically impossible to free the bran from the floury portions so nearly as when the endosperm is hard and vitreous.

It is apparent that if the yellow-berry condition were eliminated from hard wheats, the practical interests of both the grower and the miller would be subserved.

The term yellow-berry has been defined by Roberts and Freeman (6, p. 1) as—

the appearance [in hard, flinty wheats] of grains of a light yellow color, opaque, soft, and starchy. These opaque yellow grains, constituting what are called the "yellow berries," may have this character throughout; but sometime from a small fraction to half of a grain will be yellow and starchy, while the remainder of the kernel will be hard, flinty, and translucent. The difference in color between the flinty grains and the "yellow berries" is due to differences in the structure and contents of the cells of the endosperm.

The cause of yellow-berry in wheat has been the subject of some investigation. It appears to have been reported first by Bolley (2, p. 35-36), who held that the opaque, yellow spotting of the kernels was due not to heredity but to climatic factors, and that—

this peculiar mottling is due to the action of moisture, air, and sun upon the grain while it is yet in the chaff. If the weather action is long continued, the grains become evenly bleached over the entire surface. The color and hardness of the grain can be maintained by proper care in harvesting and curing.

<sup>1</sup> Reference is made by number (italic) to "Literature cited," p. 169.

<sup>2</sup> Credit is due the Department of Chemistry for the chemical analyses reported herein.

Lyon and Keyser (5, p. 25-29) came to the conclusion that—

there is quite a definite relation between the per cent of yellow berries in the crop and the character of the season in so far as the latter affects the date of ripening, the composition, and the yield of wheat.

From experimental data they find that—

the amount of "yellow-berry" increases with the lateness of ripening,  
and that—

crops of large yield and low nitrogen content contain more "yellow-berries" than do crops of the opposite kind.

They conclude that—

since it has been shown that the amount of yellow-berry increases as the ripeness of the grain increases, and also with the length of time the cut grain is exposed to the weather, it is impossible to lessen the loss by cutting the grain rather early and stacking as soon as sufficiently dry.

Roberts and Freeman (6, p. 21-35) found that in two successive years there was a diminution in the amount of yellow-berry corresponding to the shortening of the fall growing period on account of late planting. No relation was found to exist between the spring growing period and the percentage of yellow-berry, except that, in general, late ripening increased it. Higher mean temperatures for the three weeks before ripening were found to be correlated with low percentage of yellow-berry. Evidences of hereditary tendencies were found.

Headden (4, p. 30-37) studied the effects of different commercial fertilizers on yellow-berry. His results may be summarized in his own words, as follows:

In our case it is evidently the ratio between the potassium and nitrogen which determines the presence of yellow-berry. . . . The degree of mealiness or starchiness, the yellow-berry, . . . depends upon the relative available supply of these two elements. . . . The application of nitrogen, which was in the form of sodic nitrate, greatly reduced the amount of yellow-berry, in some cases preventing it altogether.

Headden does not find that climatic conditions, the soil, or the amount of available phosphorus affects the development of yellow-berry, but states that—

it can be greatly intensified or increased by the application of available potassium,  
and that—

yellow-berry indicates that potassium is present in excess of what is necessary to form such a ratio to the available nitrogen present as to be advantageous to the formation of a hard, flinty kernel. . . . I do not think that there can be any question of the identity of this affection of our wheat with that of Kansas, Nebraska, or South Dakota, and almost no question but that the opaque wheats of California and the Pacific Coast States in general are identical in their character with extreme cases of yellow-berry in Colorado and have the same cause.

This last phase of the question has not yet received much attention. The fact that yellow-berry is produced under apparently the same conditions as the flinty kernels, not merely in the same field, but on the same

plant or in the same head, indicates that yellow-berry is actually different from ordinary soft wheat.

Roberts and Freeman (6) suggested that heredity is a strong factor in determining the occurrence of yellow-berry in wheat and that pure varieties could probably be isolated that would produce little or no yellow-berry. To establish the correctness or incorrectness of this view a large number of pure strains of winter wheat were examined by the writer, and the percentage by weight of yellow-berry was determined in each.

The method pursued was as follows: From each strain of wheat, 100 cc. of grain were taken and weighed. The yellow-berry kernels were then separated and weighed. The starchy spots in the kernel almost invariably begin to appear around the germ or embryo—that is to say, at the lower end of the kernel as it stands in the glumes—and spread from there upward. In no case do the starchy spots begin to appear at the brush or tip end of the kernel. The area of the starchy spots may vary from minute dots to the entire grain. Since the opaque, starchy spots in a flinty, translucent wheat kernel may be large or small, the separation of the yellow-berry kernels must be made according to an arbitrary standard. It was decided to include as yellow-berry all kernels of which one-half or more of the grain was opaque and starchy. The starchy kernels were separated on this basis and weighed. The flinty kernels—those showing no opaque spots at all—were also separated and weighed, and the residue, if any, was designated as “neutral grains.”

The separating and weighing of the kernels was done by two persons, designated in the table by their initials, “L” and “A,” who by long experience became very expert in making the analyses of the samples. By having a part of the samples which were analyzed by one checked by the other it was found that very little difference resulted from the different individual judgments of “L,” who did the earlier, and “A,” who did the later work. It is therefore concluded that the percentage of error due to the personal equation is negligible, and that it is completely overshadowed by the positive differences in the samples themselves.

In all, 164 lots of wheat were studied, of which 77 were pure strains and 87 were checks or controls. The pure strains were grown in single rows alternately with the controls. All the rows were of the same length, 66 feet, and stood 8 inches apart. The variety used for the control rows was not a pure line but was, nevertheless, an unusually pure race of Kharkov, a standard variety long and successfully grown here. All the rows, whether of pure-line wheats or controls, contained 250 grains each, planted equidistant in the rows. The wheat was all grown in the same field, which was divided lengthwise into blocks, and each block into plots separated by narrow alleyways. The plots were all 100 by 100 feet in size. In Table I the rows are grouped according to dates of harvesting in 1908. The control row following each pure strain is given



the number of that same strain, followed by the letter "C" to indicate that it is a control. Thus, 1104 is followed by 1104 C. In cases where the numbering is not consecutive, as where a control of a different number follows a pure strain, or where no control at all is given after a pure strain, the reason lies in the fact that the omitted rows did not have the same harvesting date, or else, either through accident or winterkilling, they had been eliminated. The results of this study are given in Table I and summarized in Table II. The percentage of yellow-berry in each strain in 1907 is included in Table I so far as this information is available.

TABLE I.—Yellow-berry in different strains and varieties of hard winter wheat

Row No.	Date of harvest, 1908.	Plot No.	Percentage of yellow-berry.	
			1907.	1908.
1152 (A).....	June 24	7	3.0	4.8
1149 (A).....	do.	7	17.0	17.3
1126 (L).....	do.	7	5.0	40.4
1125 (A).....	do.	7	1.0	33.8
1066 (A).....	June 25	5	5.0	6.8
1068-4 (L).....	do.	5	.....	30.8
1068-5 (L).....	do.	5	49.0	34.0
1081-C (A).....	do.	5	.....	11.9
1122 C (A).....	do.	6	.....	27.6
1124 C (L).....	do.	6	.....	32.4
1125-C (A).....	do.	6	.....	32.8
1126-C (L).....	do.	6	.....	33.0
1128 (A).....	do.	6	9.0	24.1
1131 (L).....	do.	6	4.0	34.2
1135-C (L).....	do.	6	.....	28.2
1142-C (A).....	do.	7	.....	45.3
1145-C (L).....	do.	7	.....	32.8
1157 (A).....	do.	7	1.0	4.6
1157-C (A).....	do.	7	.....	6.8
1161 (A).....	do.	7	7.0	4.8
990 (L).....	June 26	3	1.0	11.1
991 (L).....	do.	3	.....	18.0
1000 (L).....	do.	3	2.0	16.5
1003-C (L).....	do.	3	.....	19.5
1008 (A).....	do.	4	5.0	20.5
1008 C (A).....	do.	4	.....	20.3
1009 (A).....	do.	5	5.0	5.2
1074-C (A).....	do.	5	.....	21.2
1075 (L).....	do.	5	5.0	22.2
1080 (A).....	do.	5	5.0	1.8
1080-C (A).....	do.	5	.....	19.8
1081 (A).....	do.	5	5.0	15.8
1005-C (L).....	do.	5	.....	40.7
1094 (L).....	do.	5	.....	70.4
1091-C (L).....	do.	5	10.0	28.2
1095-C (L).....	do.	5	.....	23.0
1103 (A).....	do.	6	11.0	29.7
1008 (A).....	June 27	6	.....	37.1
990-C.....	June 30	3	.....	17.9
991-C (L).....	do.	3	.....	27.0
994-C (L).....	do.	3	.....	21.0
1000-C (L).....	do.	3	.....	13.0
990-C (L).....	July 1	3	.....	29.3

TABLE 1.—Yellow-berry in different strains and varieties of hard winter wheat—Con.

Row No.	Date of harvest, 1908.	Plot No.	Percentage of yellow-berry.	
			1907.	1908.
1004-C (A).....	July 1	3		13.6
1015-C (A).....	do.	4		14.0
1036 (A).....	do.	4	10.0	56.8
1036-C (A).....	do.	4		19.0
1038 (L).....	do.	4	20.0	62.1
1038-C (L).....	do.	4		36.0
1038-1-C (L).....	do.	4		40.1
1039-3-C (L).....	do.	4		43.3
1039-4 (A).....	do.	4	5.0	23.3
1039-4-C (A).....	do.	4		23.0
1064-C (A).....	do.	4		6.2
1066-C (A).....	do.	5		12.8
1068-C (L).....	do.	5		33.9
1068-5-C (L).....	do.	5		38.0
1069-C (A).....	do.	5		12.8
1070-C (A).....	do.	5		10.1
1071 (A).....	do.	5		16.2
1071-C (A).....	do.	5		24.6
1072 (L).....	do.	5		47.0
1072-C (L).....	do.	5	1.0	44.2
1073 (A).....	do.	5		22.2
1073-C (A).....	do.	5	11.0	13.4
1075-C (L).....	do.	5		49.7
1076 (A).....	do.	5		50.9
1077 (L).....	do.	5	10.0	50.4
1077-C (L).....	do.	5	28.0	44.5
1083 (L).....	do.	5		44.9
1076-C (A).....	do.	5	4.0	18.8
1098-C (A).....	do.	6		20.2
1102 (A).....	do.	6	21.0	45.8
1103-C (A).....	do.	6		15.0
1104 (A).....	do.	6	10.0	10.7
1104-C (A).....	do.	6		11.9
1105-C (A).....	do.	6		28.9
1106 (A).....	do.	6	9.0	14.8
1106-C (A).....	do.	6		32.3
1107 (A).....	do.	6	3.0	26.8
1107-C (A).....	do.	6		19.0
1108 (A).....	do.	6	1.0	37.2
1108-C (A).....	do.	6		16.4
1109 (A).....	do.	6	2.0	37.4
1110 (A).....	do.	6	2.0	50.9
1110-C (A).....	do.	6		25.5
1111-C (A).....	do.	6		27.0
1113 (A).....	do.	6		10.0
1113-C (A).....	do.	6		26.4
1114 (A).....	do.	6		9.6
1114-C (A).....	do.	6		37.2
1115 (A).....	do.	6	1.0	29.8
1115-C (A).....	do.	6		32.3
1116 (A).....	do.	6	7.0	40.5
1116-C (A).....	do.	6		32.0
1124 (L).....	do.	6	2.0	16.5
1128-C (A).....	do.	6		16.0
1130 (A).....	do.	6		43.5
1130-C (A).....	do.	6		24.0
1132 (L).....	do.	6	20.0	80.4

TABLE I.—Yellow-berry in different strains and varieties of hard winter wheat—Con.

Row No.	Date of harvest, 1908.	Plot No.	Percentage of yellow-berry.	
			1907.	1908.
1132-C (L).....	July 1	6		69.0
1135 (L).....	do.	6	21.0	86.0
1140 (L).....	do.	6	2.0	94.5
1140-C (L).....	do.	6		66.9
1145 (L).....	do.	7	25.0	99.0
1150-C (A).....	do.	7		10.3
1151 (A).....	do.	7	1.0	8.8
1151-C (A).....	do.	7		12.9
1152-C (A).....	do.	7		11.4
1154 (L).....	do.	7	6.0	75.3
1154-C (L).....	do.	7		41.7
1160 (L).....	do.	7		49.0
1161-C (A).....	do.	7		10.3
1162 (A).....	do.	7	5.0	62.4
1162-C (A).....	do.	7		11.4
1163 (L).....	do.	7	12.0	69.1
1163-C (L).....	do.	7		28.3
1164-1 (L).....	do.	7	23.0	45.3
1164-1-C (L).....	do.	7		34.6
1164-2 (L).....	do.	7	30.0	64.8
1372-1 (A).....	do.	7	2.0	40.3
1372-1-C (A).....	do.	7		15.2
1372-8-C (A).....	do.	7		14.1
1117-C (L).....	do.	7		25.8
1160 (L).....	July 3	7	30.0	61.5
1164-2 C (L).....	do.	7		52.6
1058-6 (L).....	do.	8		26.7
1058-6-C (L).....	do.	8		20.2
1117 (L).....	do.	8		53.0
1138-C (A).....	do.	8		8.1
1050-7 (L).....	do.	1		45.4
1050-7-C (L).....	do.	1		45.2
1067 (L).....	do.	1	17.0	87.0
1067-C (L).....	do.	1		41.5
1061 (L).....	do.	1		90.2
1061-C (L).....	do.	1		43.4
1146 (L).....	do.	1	32.0	90.4
1146-C (L).....	do.	1		59.7
1147-C (L).....	do.	1		78.4
1003 (L).....	July 6	3	10.0	15.0
1004 (A).....	do.	3		59.8
1058-1 (L).....	do.	4		32.5
1058-5 (A).....	do.	4	1.0	20.3
1059-3 (L).....	do.	4	1.0	38.9
1059-6 (L).....	July 10	1		30.0
1059-6-C (L).....	do.	1		38.0
1002-C (A).....	do.	1		33.7
1039 (L).....	do.	1		67.9
1039-C (L).....	do.	1		65.0
1068-1-C (A).....	do.	1		26.4
1066-C (A).....	do.	1		28.3
1139 (L).....	do.	1		92.2
1139-C (L).....	do.	1		79.5
1147 (L).....	do.	1	4.0	94.4
1158 (L).....	July 12	7	1.0	65.5
1158-C (L).....	do.	7		38.8

TABLE II.—Summary of yellow-berry in hard winter wheat, 1908

Date of harvesting, 1908.	Total number of cases.	Number of pure strains.	Percentage of yellow-berry.	Number of controls.	Percentage of yellow-berry.	Average percentage of yellow-berry.
June 25.....	16	7	20	9	28	24
26.....	17	10	21	7	25	23
30.....	4	0	.....	4	20	20
July 1.....	82	33	45	49	26	34
3.....	15	7	65	8	45	54
6.....	5	5	33	0	.....	33
10.....	10	4	71	6	45	55

Tables I and II give the results for 73 pure strains and 83 controls out of the total number of 77 and 87, respectively, that were originally planted. The average percentage of yellow-berry in the control plots is given in Table III.

TABLE III.—Average percentage of yellow-berry in control plots, 1908

Plot No.	Number of rows.	Percentage of yellow-berry.
3.....	7	20.2
4.....	8	26.0
5.....	17	26.3
6.....	22	29.5
7.....	15	24.5
8.....	3	21.0
Average.....	.....	26.1

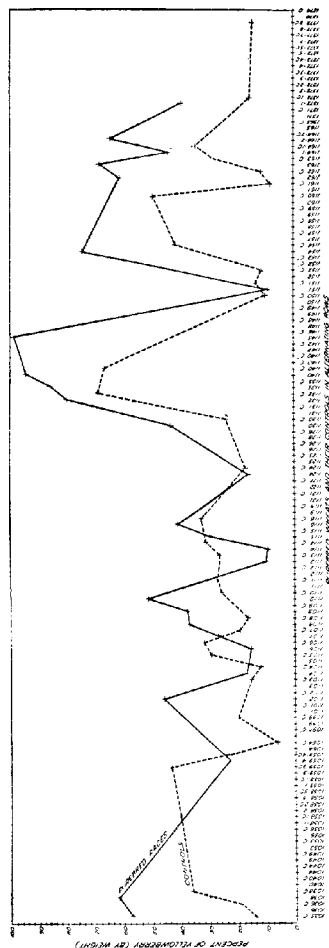
From this table it appears that the control rows were quite constant in their tendency to produce yellow-berry. However, as shown in figure 1, there is considerable variation in the amount of yellow-berry in individual rows. The general trend of the percentage of yellow-berry in the pure strains follows that of the controls. This indicates that the differences depend upon the same causes in the pure strains as in the controls, and that the changing conditions in different parts of the plot had more influence in causing an increase or a decrease in yellow-berry than did any hereditary factors.

The yield per row presents a similar phenomenon. There is a greater variability in the yield of the individual rows in the pure strains than in the control rows, but the general upward and downward trend of the two curves coincides very closely. This would indicate that external conditions in the plots were more important than varietal characteristics in determining both the yield and the percentage of yellow-berry.

An inspection of Table I shows that there are a number of cases in which there is apparent coincidence between the percentage of yellow-berry

produced in one season and the percentage produced by the same strain the following season. However, a correlation table between yellow-berry percentages for the two successive seasons plotted for 56 strains that were planted and harvested on the same date gives a correlation coefficient of only  $0.078 \pm 0.005$ . This extremely low correlation indicates that the external conditions are the determining factors to a degree which the hereditary tendencies of the plant have little power to modify. On the other hand, a graph showing the percentage of yellow-berry in 1907 and 1908 for those strains having 10 per cent or more of yellow-berry indicates that there is a hereditary relation; and were the number of cases larger, distinct indications of inheritance would be seen.

FIG. 1.—Percentage by weight of yellow-berry kernels in pure-bred wheats and their controls, 1908.



conclusions with respect to these dates. It appears, however, that in the season of 1908 there was a close relation between the percentages of

yellow-berry and the date of ripening. This is in harmony with the general assumption that a longer growing and a slower ripening period produces yellow-berry.

#### PHYSICAL CHARACTERS OF YELLOW-BERRY

Lyon and Keyser (5, p. 32) cite Nowacki to the effect that—the difference between mealy and horny wheat kernels is due to the presence in the former of a larger volume of air spaces than in the latter. He urges that the vacuoles

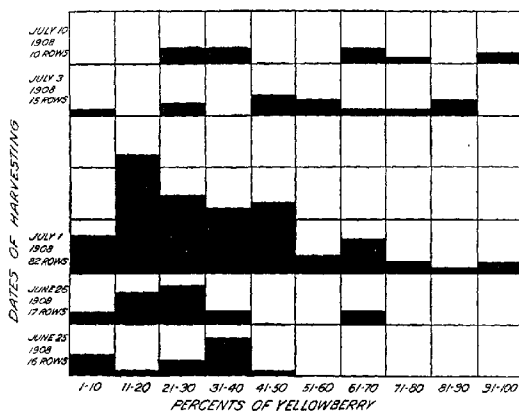


FIG. 2.—Relation between date of harvesting and percentage of yellow-berry.

that occur in the protoplasm of the cell decrease in size and number as the endosperm develops, and that the more protoplasm, the smaller and fewer the vacuoles.

Lyon and Keyser (5, p. 34) found that—

a typical mealy wheat like the soft, white Sonora of California contained starchy granules measuring from 0.02817 millimeters in diameter for the larger, to 0.005634 millimeters for the smaller. A typical horny Turkish Red kernel contained starch grains varying between the extremes of 0.014085 and 0.002817 millimeters in diameter. A typical yellow-berry Turkish Red kernel showed larger starch granules, 0.017042 millimeters for the larger, and 0.003081 millimeters for the smaller sizes.

Cobb (3, p. 572) found that—

it is noticeable that when the grain is rich in nitrogenous matter the number of large starch granules is smaller. As we pass in such grains in our examination from the center to the outside, we note a gradual decrease in the size of the starch granules, and even at some little distance from the aleuron layer the cells are filled with small granules only.

Lyon and Keyser's examination (5, p. 35) of horny and starchy kernels revealed more numerous and larger vacuoles in the latter, with only an

occasional vacuole in the former. It is stated that large starch granules and large or numerous vacuoles are associated in starchy kernels, and that—the difference in structure between the horny and the yellow kernels is also accompanied by a difference in composition, the yellow kernels containing less nitrogen.

The size of the starch granules in yellow-berry and in hard, flinty wheat was studied by the writer. Yellow-berry kernels were taken from a number of pure wheats, and samples of the opaque or yellow portions of the endosperm were removed from these by means of a dental drill. Samples were taken from 10 kernels to get a fair average. Similar samples were taken from the horny or flinty portion of the same 10 kernels, the drill being burned off each time after use.

The yellow-berry endosperm samples were shaken up in alcohol, stained with iodine in potassium iodide, and mounted for measurement with a Bausch and Lomb filar micrometer. Five hundred measurements were made for each 10-grain sample of each strain of wheat used. In all cases, the largest starch granules visible in any given field were the ones chosen for measurement. The results of this study are given in Table IV.

TABLE IV.—Measurements of starch grains from yellow-berry and from hard kernels of 10 pure lines of wheat

Sample No.	Measurement of starch granules in—		Difference between soft and hard grains.
	Hard grains.	Soft grains.	
	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>
951.....	0.030726	0.024819	—0.005907
1094.....	.024999	.029095	+ .004096
1119.....	.025910	.023940	— .001970
1126.....	.027315	.024977	— .002338
1150.....	.032587	.027681	— .004906
1519-8.....	.027873	.028878	+ .001005
1592-6.....	.028234	.028069	+ .000165
1687-1.....	.020413	.027965	— .007552
1687-8.....	.031869	.023822	— .008047
1687-10.....	.033200	.028519	— .004681
Average.....	.029212	.026897	— .002315

These results show that in 7 out of 10 cases the average diameter of the starch grains in the hard portions of the kernels was greater than in the soft or yellow-berry portions, while in 3 cases it was less. These results are exactly the reverse of those obtained by Lyon and Keyser (5, p. 23-26).

The writer is unable to account for the discrepancy in the two sets of data. It would seem, however, that since in the present case an average of 500 measurements was taken and the largest starch grains in each

microscopic field were measured, fairly uniform and accurate results have been obtained.

Table V shows the frequency of distribution of the starch grains with respect to size, expressed in micromillimeters (microns) in nine of the races or pure strains just considered. From this table it appears that, in both the hard kernels and the yellow grains, the greatest number of individual cases (the mode) falls into the class of 25 to 29.9 micromillimeters diameter, although the average size of the starch grains in the hard kernels was about 2.3 microns greater than in the yellow-berry kernels.

TABLE V.—Distribution of starch grains of yellow-berry and hard kernels with respect to size

Pedigree No.	Diameter of starch grains expressed in micromillimeters ( $\frac{\text{mm.}}{1000}$ )											
	Number of starch grains having diameter of—											
	14.9	19.9	24.9	29.9	34.9	39.9	44.9	49.9	54.9	59.9	64.9	74.9
951 h.....	26	118	121	90	78	31	22	14				
951 Y.....	46	235	172	40	5	1						
1094 h.....	1	63	207	167	46	16	7	1				
1094 Y.....	9	87	198	154	45	7						
1119 h.....	36	197	174	74	19							
1119 Y.....	69	259	136	33	2	1						
1126 h.....	7	133	245	97	18	3						
1126 Y.....	40	209	196	46	3							
1516-8 h.....	5	124	224	118	26	3						
1516-8 Y.....	6	72	218	180	23	1						
1592-6 h.....	15	127	179	124	47							
1592-6 Y.....	6	71	227	162	29	5						
1687-4 h.....	5	32	88	147	117	65	22	8	2	0	1	
1687-4 Y.....	5	46	119	166	90	14	23	6	2			
1687-8 h.....	17	42	57	93	83	71	79	23	5	2	1	1
1687-8 Y.....	2	115	220	127	46	13	2					
1687-10 h.....	9	27	64	90	108	83	51	25	19	7	3	2
1687-10 Y.....	10	39	104	150	108	61	26	6	2			
Total, hard.....	32	253	1,125	1,449	857	426	193	79	40	9	5	3
Total, yellow.....	17	382	1,376	1,596	859	224	66	12	4			

Other physical characteristics of the yellow-berry and hard kernels—for example, specific gravity, average kernel-weight, and volume-weight, are given in Table VI for 10 strains of wheat used for the study of the size of starch granules. The volume-weight, the test weight per bushel, and the average weight per kernel is higher for the yellow-berry than for the hard kernels. The specific gravity is somewhat higher in the hard wheat. These results agree in general with those previously reported by the writer (6), except that in the earlier investigation the average weight per kernel was higher for the hard wheat.

Snyder (7, 8) has investigated the comparative weight of light and dark seeds taken from the same samples of varieties from 31 miscellaneous



sources, and of 32 varieties grown from selected seed. Most of these were Minnesota-grown. The average weight per kernel was higher for the dark grains in one case and for the light grains in the other.

TABLE VI.—*Specific gravity, kernel-weight, and volume-weight of hard (h) and yellow-berry (y) wheat*

Sample No.	Specific gravity.	Average weight per kernel.	Volume weight.	Test weight.
		Gm.	Gm. per 100 cc.	
951 Y.....	1.368			
	1.367	0.029	83.42	64.76
951 h.....	1.387			
	1.378	.027	80.85	62.80
1094 Y.....	1.351			
	1.362	.031	80.00	62.15
1094 h.....	1.395			
	1.385	.027	79.63	61.86
1119 Y.....	1.380			
	1.373			
1119 h.....	1.399			
	1.399	.031	79.00	61.37
1126 Y.....	1.388			
	1.360	.025	83.72	64.59
1126 h.....	1.400			
	1.411	.028	82.22	63.44
1150 Y.....	1.379			
	1.379	.033	79.12	61.46
1150 h.....	1.379			
	1.379	.033	77.12	60.33
1516-8 Y.....	1.367			
	1.367	.029	82.88	63.95
1516-8 h.....	1.387			
	1.390	.031	78.66	60.69
1592-6 Y.....	1.345			
	1.347	.026	77.04	59.44
1592-6 h.....	1.368			
	1.381	.022	77.23	59.59
1687-4 Y.....	1.373			
	1.370	.030	82.05	63.31
1687-4 h.....	1.401			
	1.401	.032	79.29	61.18
1687-8 Y.....	1.372			
	1.378	.029	79.40	61.68
1687-8 h.....	1.390			
	1.393	.029	78.88	61.28
1687-10 Y.....	1.377			
	1.370	.028	80.00	62.15
1687-10 h.....	1.391			
	1.386	.027	75.13	58.38
Average, Y.....	1.369	.0291	80.66	62.49
Average, h.....	1.392	.0287	78.73	61.00

Stewart and Hirst (10) and Stewart and Greaves (9), of the Utah Agricultural Experiment Station, found in comparing the average weight of kernels of a considerable number of hard winter wheats, semihard winter wheats, and soft winter wheats, that soft wheat varieties had the heaviest kernels and hard wheats the lightest.

## THE CHEMICAL COMPOSITION OF YELLOW-BERRY WHEAT

The chemical composition of yellow-berry, especially as related to protein content, has been the subject of several investigations. Snyder, of the Minnesota Experiment Station (7, 8), in comparing 63 light (starchy) and 30 dark (flinty) lots of grain, found a slight difference in the protein content in favor of the hard grain. The chief differences in chemical composition of yellow-berry and hard kernels of the same sample found by the writer are a higher moisture content, a lower protein content, and a higher starch content in the yellow-berry kernels as compared with the hard kernels. The data secured in this investigation are given in Tables VII and VIII.

TABLE VII.—Analyses of yellow-berry wheat

Chemistry laboratory No.	Botany laboratory No.	Moisture.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Pentosans.	Starch.	Ether extract.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
603.....	911	8.47	1.81	10.16	2.21	74.99	7.90	66.34	1.84
605.....	1094	8.43	1.86	10.48	2.23	75.68	7.66	67.65	1.94
607.....	1119	8.59	1.71	10.44	2.19	75.29	7.77	64.50	1.78
609.....	1126	7.37	1.64	9.72	2.10	77.64	7.68	67.33	2.13
612.....	1150	7.71	1.95	10.26	2.05	75.51	7.99	66.17	1.90
613.....	1516-8	7.71	1.57	10.80	2.19	75.68	7.68	70.62	2.05
615.....	1592-6	7.73	1.71	10.51	2.53	76.04	8.03	63.47	1.95
617.....	1687-4	7.21	1.87	10.80	2.15	75.92	7.85	65.16	2.05
619.....	1687-8	6.71	1.94	12.79	2.30	76.15	7.53	65.46	2.10
621.....	1687-10	8.07	1.79	10.96	2.22	74.65	7.67	68.18	2.00
Total.....		78.46	17.88	105.15	22.45	756.77	75.36	670.10	19.74
Average.....		78.46	17.88	105.15	22.45	756.77	75.36	670.10	19.74

TABLE VIII.—Analyses of hard, flinty wheat

Chemistry laboratory No.	Botany laboratory No.	Moisture.	Ash.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Pentosans.	Starch.	Ether extract.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
604.....	911	8.20	1.99	12.00	2.55	73.11	8.17	58.52	1.85
606.....	1094	8.61	2.03	12.00	2.70	73.02	7.80	60.93	1.81
608.....	1119	7.97	2.06	12.00	2.36	73.93	7.12	66.95	1.65
610.....	1126	7.41	1.91	11.37	2.33	75.10	7.87	61.96	1.93
612.....	1150	7.68	2.01	12.30	2.11	74.96	8.17	64.80	1.82
613.....	1516-8	7.22	1.87	13.08	2.18	74.55	7.85	61.79	2.10
616.....	1592-6	7.20	1.90	12.04	2.50	74.35	8.12	62.01	1.85
618.....	1687-4	6.98	1.82	12.12	2.19	74.96	7.66	61.99	1.95
620.....	1687-8	7.31	2.02	12.65	2.35	74.14	7.53	61.67	1.97
622.....	1687-10	7.83	2.04	11.96	2.49	73.80	7.93	60.81	1.88
Total.....		76.34	19.66	119.90	23.66	711.61	78.83	647.62	18.83
Average.....		76.34	19.66	119.90	23.66	711.61	78.83	647.62	18.83

The results show a higher percentage of starch in the yellow-berry wheat than in the flinty kernels, the average percentages being 67.01 and 61.762, respectively. They show also an average starch ratio of 6.37 for the yellow-berry kernels and of only 5.40 for the flinty kernels. It appears probable that the smaller amount of protein in the starchy grains is not only fully compensated for by an equivalent deposition of starch but more than compensated for, since the percentage of protein is 1.475 less and the percentage of starch 2.248 greater in the starchy than in the flinty kernels.

## SUMMARY

(1) This investigation is a continuation of the work reported in Kansas Agricultural Experiment Station Bulletin 156.

(2) The opaque, starchy spots in wheat kernels which give them the designation of yellow-berry kernels almost invariably begin to appear in the neighborhood of the germ or embryo, the lower end of the kernel as it stands on the plant, and spread from there upward.

(3) One hundred and sixty-four lots of wheat were investigated to determine the relation of yellow-berry to field conditions, especially the period between first heading and ripening. Seventy-seven of these lots were pure strains or pure lines, and 87 were checks or controls.

(4) In determining the percentage of yellow-berry, an arbitrary standard was adopted. If one-half or more of a kernel was opaque it was weighed as a yellow-berry kernel. The flinty kernels free from opaque portions were weighed separately, and the residue of the kernels were designated as neutral grains.

(5) The variation in yellow-berry percentages in the yields of the control rows was closely followed by that of the pure-line rows alternating with them. The general trend of the whole series of the pure lines follows that of the controls.

(6) The conclusion from the field tests is that the operation of common causes for the production of yellow-berry overshadowed any differences that may have been due to hereditary tendencies, and precludes a definite statement regarding the relation of hereditary tendencies in hard winter wheats toward the production of yellow-berry. That some isolated pure strains of wheat are freer from yellow-berry than others growing in the same field and under apparently identical conditions of soil and climate is, however, possible.

(7) With respect to the relation of yellow-berry to date of ripening, the experiment shows a higher percentage of yellow-berry with the later dates of ripening.

(8) The comparative size of the starch granules in yellow-berry and in flinty grains was investigated, 500 measurements of starch grains being made from hard and from yellow-berry samples of 10 strains of pure-line wheats. The largest starch grains in the yellow-berry portions of the kernel were found to be smaller on the average than the largest starch grains in the flinty portions of the same kernels. These results seem to contradict those of Cobb and of Lyon and Keyser.

(9) In respect to the average kernel-weight, the yellow-berry kernels were found to weigh on the average 0.4 mg. more than the flinty kernels, based on the average weight (air-dried at 100° C.) of 100 kernels. In an earlier study the flinty kernels were on the average 1.4 mg. heavier.

(10) In specific gravity the flinty kernels were found to be 0.0230 heavier than the yellow-berry kernels.

(11) The yellow-berry kernels were found to be higher in moisture and starch content and lower in protein and ash than the hard, flinty kernels.

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# NOTE ON THE EUROPEAN CORN BORER (*PYRAUSTA NUBILALIS* HÜBNER) AND ITS NEAREST AMERICAN ALLIES, WITH DESCRIPTION OF LARVÆ, PUPÆ, AND ONE NEW SPECIES

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The introduction of the European corn borer (*Pyrausta nubilalis* Hübner) into certain sections of Massachusetts and New York and the possibility of its wider distribution has necessitated a careful study of the larva of this dangerous pest, particularly as the larva of a native *Pyrausta* also attacks corn, has much the same habits as that of *P. nubilalis*, and so closely resembles it that the two are easily confused. The adult females of the two species are also very similar, and to any but a specialist familiar with the group they are certain to cause difficulty. For this reason it is desirable to have full descriptions of adults, pupæ, and larvæ which will enable positive identification and will separate *P. nubilalis* from its nearest American allies. The present paper is presented with this object.<sup>2</sup>

## PYRAUSTA

### GENERAL CHARACTERS

ADULT (PL. 7, A, B, E, F; 8, B, C)

Ocelli present. Proboscis developed. Labial palpi developed; porrect; triangularly scaled; third joint hidden by hair. Maxillary palpi present; slightly dilated at apex. Frons rounded. Antennæ three-fourths; finely ciliated. Tibiæ smooth-scaled; outer spurs short; outer medial spur not more than two-thirds the length of the inner. Forewing with 12 veins; 1c absent; 1a separate from 1b; 1b simple; 2 from before angle of cell; 3, 4, 5 from lower angle of cell; 6 from near upper angle of cell; 7 from the cell, to termen, almost straight; 8 and 9 stalked; 10 closely approximate with 8 and 9. Hindwing as broad as forewing; frenulum present, single in male, multiple in female; median vein nonpectinate on upper side; 8 veins; 1a,

<sup>1</sup>For material necessary for these studies the writer is indebted to Messrs. W. R. Walton, D. J. Caffrey, and Geo. G. Ainslie, of the Bureau of Entomology, especially to the latter, who has furnished reared series of moths of *P. umsliei* and *P. penitalis* and a quantity of larvæ and pupæ of both species. In the United States National Museum, aside from this, there are bred series of *P. penitalis* and *P. umsliei* from various localities.

Dr. L. O. Howard has also kindly provided authentic European larvæ, blown and alcoholic, of *P. nubilalis*, secured through the courtesy of Prof. F. L. Bouvier, of Paris, France.

For the drawings accompanying this paper the writer is indebted to Miss E. Hart and Miss Ada P. Kneale, of the Bureau of Entomology. Miss Kneale has contributed figures of the female genitalia on Plate 8. The rest are by Miss Hart.

<sup>2</sup>Since these studies were begun, a suggestive paper dealing with the larvæ of *P. nubilalis* and other lepidopterous borers has appeared. (MOSHER, Edna. NOTES ON LEPIDOPTEROUS BORERS FOUND IN PLANTS, WITH SPECIAL REFERENCE TO THE EUROPEAN CORN BORER. *IN JOUR. ECON. ENT.*, v. 12, no. 3, p. 238-268. 1919.)

rb, 1c present; 1b simple; 3, 4, 5 from lower angle of cell; 6, 7 from upper angle; 7 anastomosing with 8 beyond cell. Male genitalia with uncus rudimentary or absent; transtilla present; harpes with prominently developed clasper.

PUPA (PL. 9, C-F)

Moderately slender; abdominal segments gradually tapering; smooth except for a slight rugosity on dorsum and a single row of 4 or 5 short spines on dorsum of abdominal segments 1 to 7; wings extending to or nearly to ventro-caudal margin of fourth abdominal segment; cephalic end bluntly rounded, tapering from mesothorax; epicranial suture present, represented by a straight line; vertex distinct, rather narrow; labrum, pilifers, and maxillary palpi well developed; labial palpi small; prothoracic and mesothoracic legs not extending cephalad between sculptured eyepiece and antenna; maxillae long, extending nearly the length of the wings; femora of prothoracic legs clearly indicated; prothoracic legs extending half the length of the wings; mesothoracic and metathoracic legs extending to the tips of the wings; antennae extending nearly the length of the wing; proleg scars plainly visible on abdominal segments 5 and 6; mesothoracic spiracle with a strongly chitinized caudal ridge, without setae; abdominal spiracle slightly produced; anal and genital openings slitlike in both sexes; cremaster present, prominent, stout, spatulate, and armed at extremity with a cluster of 4 or 5 short curled hooks.

LARVA (PL. 10, A-D; 11, A, D-H)

Cylindrical; moderately stout; abruptly tapering at caudal end. No secondary hair. Legs and prolegs normal. Crochets triordinal, in a circle broken outwardly. No anal fork. Prothoracic shield moderately broad, divided. Spiracles oval, moderate; that on eighth abdominal segment slightly higher than those on abdominal segments 1 to 7; no more than  $1\frac{1}{2}$  times as large; same size as that on prothorax. Skin covered with fine granulations (PL. 11, E, F) especially strong and dense on dorsum, diminishing toward venter and absent in folds marking the body areas and a small space about the chitinized tubercles.

Body setae moderately long; tubercles prominent, broadly chitinized; IV and V on abdominal segments 1 to 8 under the spiracle and approximate; prespiracular shield of prothorax small or moderate, nearly square, bearing only two setae (IV and V) situated ventro-cephalad of the spiracle, III of prothorax absent; group VI bisetose on prothorax, unisetose on mesothorax and metathorax; IV and V united on abdominal segment 9 and approximate to III; III directly in front of the spiracle on abdominal segment 8, over the spiracle on abdominal segments 1 to 7; III<sup>a</sup> present; group VII trisetose on abdominal segments 1 to 6, bisetose on abdominal segment 7, unisetose on abdominal segments 8 and 9; abdominal segment 9 with all setae in a vertical line, 1 absent; on abdominal segments 1 to 8, II is latero-caudad of I; prothorax with II<sup>a</sup> higher than I<sup>a</sup>, dorso-caudad and remote from II<sup>b</sup>, closer to I<sup>a</sup> than to II<sup>b</sup>, II<sup>b</sup> on the level of puncture 2; I<sup>c</sup> equidistant from I<sup>a</sup> and puncture 2, punctures x and y dorso-caudad of I<sup>a</sup>, distance between I<sup>a</sup> and II<sup>a</sup> slightly greater than between I<sup>a</sup> and I<sup>c</sup>.

Head capsule spherical, nearly square in outline viewed from above, slightly wider than long; greatest width at middle of head; incision of dorsal hind margin not over one-fourth the width of the head; distance between dorsal extremities of hind margin less than one-half the width of the head; from dorsum of antennal ring a slight projection of the epicranium forming an antennal shield (ATS). Frons broad, as long as or a trifle longer than wide, reaching beyond middle of head. Adfrontal sutures extending to incision of dorsal hind margin. Longitudinal ridge (LR) short, less than one-half the length of the frons.

Ocelli six; lenses well defined.

Epistoma normal.

Frontal punctures close together; very slightly forward of frontal setae; distance between punctures less than distance from puncture  $P^a$  to setae  $L^1$ ; distance from frontal setae ( $P^1$ ) to first adfrontal seta ( $AdF^1$ ) about equal to distance separating adfrontal setae ( $AdF^1$  and  $AdF^2$ );  $AdF^2$  approximate to beginning of longitudinal ridge; puncture  $AdF^a$  about equidistant from  $AdF^1$  and  $AdF^2$ .<sup>1</sup>

Epicranium with the normal number of primary setae and punctures and with the three ultraposterior setae and one ultraposterior puncture distinguishable. Anterior setae ( $A^1$ ,  $A^2$ ,  $A^3$ ) forming a slightly obtuse angle; anterior puncture ( $A^a$ ) posterior to seta  $A^2$ . Posterior setae ( $P^1$  and  $P^2$ ) and puncture  $P^a$  about middle of head;  $P^1$  nearly on the level of lateral seta ( $L^1$ ), behind the level of  $AdF^1$ ;  $P^2$  behind the level of place of juncture of adfrontal ridges; posterior puncture  $P^a$  approximate to lateral seta ( $L^1$ ); posterior puncture  $P^b$  lying between  $P^1$  and  $P^2$  approximate to  $P^2$ ;  $P^1$ ,  $P^2$ ,  $P^a$  and setae and puncture of ultraposterior group forming nearly a straight line with frontal seta ( $F^1$ ). Lateral seta ( $L^1$ ) well forward on head but not closely approximate to  $A^1$ ; lateral puncture posterior or postero-ventrad to  $L^1$ , remote. Ocellar setae ( $O^1$ ,  $O^2$ ,  $O^3$ ) well separated;  $O^1$  ventrad of ocelli II and III, approximate to ocellus III;  $O^2$  ventrad or postero-ventrad of ocellus I;  $O^3$  directly ventrad of  $O^2$ , remote, further from  $O^2$  than  $O^1$  is from  $O^2$ ; ocellar puncture ( $O^a$ ) approximate to ocellus VI. Subocellar setae ( $SO^1$ ,  $SO^2$ ,  $SO^3$ ) triangularly placed; puncture  $SO^a$  nearer to  $SO^2$  and  $SO^3$  than to  $SO^1$ . Genal puncture ( $G^a$ ) anterior to the seta ( $G^1$ ).

Labrum with median incision broadly triangular, moderately deep; median setae ( $M^1$ ,  $M^2$ ,  $M^3$ ) triangularly placed;  $M^2$  postero-laterad of  $M^1$  and considerably closer to  $M^1$  than to  $M^3$ ;  $La^1$  directly laterad of and closely approximate to  $La^2$ ;  $La^1$  and  $La^2$  on the level of  $M^1$ ;  $La^2$  and  $M^3$  on the same level, rather well back of anterior margin of labrum; puncture approximate and posterior to  $M^2$ .

Epipharyngeal shield narrowly bordering the greater part of median incision of labrum. Epipharyngeal setae triangularly grouped; well separated and well behind anterior margin of epipharynx; narrow, moderately long. Epipharyngeal rods indicated only by their prominent posterior projections.

Maxillulae normal; the large lateral lobes heavily spined but without blades or distinctly modified setae.

#### PYRAUSTA NUBILALIS

*Pyrausta nubilalis* Hübner, 1901. in Staud. and Rebel, Cat. Lepidop., Aufl. 3, Bd. 2, p. 65, No. 1218.

#### ADULT

MALE.—Underside of palpi snow white; palpi otherwise grayish fuscous. Head and thorax grayish fuscous. Forewing dark grayish fuscous; transverse antemedial and transverse postmedial lines outwardly margined with bright ochreous which, in the latter, broadens out to a distinct blotch at tornus; area between obicular and discal mark bright ochreous; at base of inner margin a distinct oval patch of firmly attached semimetallic brown sex scaling under surface scaling of the wings. Hindwing dark grayish fuscous; a broad, pale ochreous postmedian fascia not extending completely to dorsum. Abdomen dark grayish fuscous above; posterior margins of segments edged with a fine line of white scales. Genitalia (Pl. 7, A) as figured; apex of tegumen shortly trifurcate; anellus with two long, slender, dorsally projecting arms (anellus lobes); harpe with three stout spines arising from inner margin of sacculus at fusion with base of clasper; face of clasper oval, somewhat kidney-shaped. Alar expanse, 20 to 26 mm.

<sup>1</sup> There are some individual variations and considerable asymmetry in different specimens of the same species in the position of the adfrontal setae and puncture and also in the length of the longitudinal ridge. The setae will not always be on the same level on both sides of the head, and in some specimens  $AdF^a$  will be slightly nearer to  $AdF^1$  than to  $AdF^2$ ; but aside from such individual variations, which are common among lepidopterous larvae and for which allowance must always be made, the characters hold remarkably well.



**FEMALE.**—Darker portion of palpi, head, and thorax pale or dull creamy ochreous. On forewing outer margins of transverse antemedial and transverse postmedial lines, terminal margin, and area between obicular and discal mark whitish ochreous. Post-medial fascia of hindwing whitish ochreous. Darker portions of forewing and hindwing pale gray, grayish ochreous, or ochreous gray tinged with ferruginous. Genitalia as figured (Pl. 8, A, B); genital opening without strong chitinous anterior margin; chitinized plate, posterior to genital opening, well developed, nearly square. Alar expanse, 18 to 34 mm.

#### PUPA

Fourteen to 16 mm. long; yellowish brown, darker towards extremities, cephalic end blackish brown; thorax but slightly humped; abdominal spiracles small, oval. Edges strongly chitinized, blackish brown; front smooth; cremaster (Pl. 9, F) longer than broad.

#### LARVA

Full grown 23 to 25 mm. long by 3 to 3.5 mm. broad. Body sordid white, shading to smoky fuscous on heavily granulose dorsal and lateral areas; smoky color forming a distinct, broad, longitudinal band along entire dorsum with a more distinct and darker narrow central band; creases of folds and areas immediately surrounding chitinized tubercles clear white; above and behind abdominal seta III and before abdominal seta I some of the muscle attachments are indicated by lines or clusters of white spots more or less fused.<sup>1</sup>

Chitinized areas of the body strongly pigmented; thoracic shield light yellow, laterally and caudally bordered by a narrow band of smoky fuscous and more or less spotted with brownish, above seta II<sup>o</sup> the spots fusing into one or two more or less extended and conspicuous splotches; anal shield yellow, irregularly spotted, especially near margins, with smoky fuscous; chitinized areas of tubercles moderately large, irregularly oval or circular, yellow with a more or less extended border of smoky fuscous, which sometimes on those above the spiracle covers the entire tubercle; tubercle I of abdomen with one or two fuscous spots cephalo-laterad of the seta; dorso-caudad of the spiracle on proleg bearing abdominal segments 3 to 6 a small, chitinized, brownish, thornlike projection (Pl. 11, G, mt), quite plain in some specimens.<sup>2</sup> Setae brownish at base, pale towards tip, slender. Thoracic legs yellow; claws brown. Crochets of prolegs unevenly triordinal; 32 to 46 (averaging 40); brown. Spiracles broadly oval; chitinous ring light brown.

Head brown, more or less mottled with blackish, in some specimens giving the whole head a blackish brown appearance; ocellar pigment black and in the form of a band under the ocelli, continuous. Anterior setae A<sup>1</sup> and A<sup>2</sup> and puncture A<sup>6</sup> in a line or with A<sup>6</sup> a trifle postero-laterad of A<sup>2</sup>, not postero-dorsad; A<sup>2</sup> somewhat nearer to A<sup>3</sup> than to A<sup>5</sup>, A<sup>1</sup>, A<sup>2</sup>, and A<sup>3</sup> forming a decided obtuse angle; ocellar puncture O<sup>3</sup> closely approximate and directly posterior to ocellus VI. Labium and maxillae as figured; no decided hump in shoulder of stipes maxillaris; chitinized area of palpi maxillaris yellow, strongly shaded with black. Mandible five-toothed; nearly square; distal tooth small and pointed; median edge outwardly angulated.

<sup>1</sup> These are the clear spaces referred to by Miss Mosher (op. cit.) and one of the characters which she uses to distinguish *Pyrausta nubilalis* from the so-called *P. penialis* (*andus*). The writer has been unable to find any real, consistent difference in this character between the two species. In some forms (particularly certain *Phycitinae*—*Dioryctria* and *Pinipestis*, for example) the points of attachment of the muscles are pigmented and slightly chitinized, forming a series of dark-colored pits, which are quite characteristic. Here only a few of the attachments are indicated by pits or spots, and these are colorless and more or less lost in the clear spaces of the folds indicating the limits of the body areas.

<sup>2</sup> Miss Mosher (op. cit.) refers to this structure as a sensory pore. It is in fact merely a chitinous support at the point of attachment of one of the strong proleg muscles and similar to the chitinization in the center of the proleg itself.

## PYRAUSTA AINSLIEI

*Pyrausta ainsliei* n. sp.*Pyrausta penitalis* Authores (nec. Grote).

Underside of palpi near base snow white; palpi otherwise yellow. Head and thorax yellow. Forewings pale yellowish with very slight dusting of darker cream yellow without the distinctly ferruginous powdering of *P. penitalis*; transverse antemedial and transverse postmedial lines as in *P. penitalis*; darker shading beyond transverse postmedial line faint; obicular marking as in *P. penitalis*; the dusky blotch beyond the cell reduced to a mere shading, scarcely distinguishable; terminal margin and cilia pale yellow; no sex scaling at base of inner margin of forewing of male. Hindwing as in *P. penitalis* except more distinctly marked than the pale forms of the latter species and lacking the ferruginous-ochreous margins of the small dark *P. penitalis*. Male genitalia as figured (Pl. 7, C); apex of tegumen rounded; anellus with two long, slender, dorsally projecting arms (anellus lobes); harpe with two or three stout spines arising from inner margin of sacculus at fusion with base of clasper; face of clasper triangular. Female genitalia as figured (Pl. 8, E, F), with genital opening strongly chitinized anteriorly. Alar expanse, 20 to 27 mm.

HABITAT.—Knoxville, Tenn., type locality (Ainslie and Cartwright); Arlington and Woodburn, Mass. (D. J. Caffrey); Milford, Conn. (M. P. Zapp); Hopewell Junction, N. Y.; Oak Station, Pa. (Fred Marloff); Plummer's Island, Md. (R. P. Currie); Tryon N. C. (W. F. Fiske); Missouri; Maine; St. Johns, Quebec (W. Chagnon).

FOOD PLANTS.—Polygonum, Ambrosia, Xanthium, Eupatorium, corn.

TYPE.—Cat. No. 32544, U. S. N. M.

*P. ainsliei* was described from one male type and eight male and seven female paratypes. It was named in honor of George C. Ainslie, of the Bureau of Entomology, who has made a special study of the life history and to whom the author is indebted for material and information on its habits. This is the species that has appeared in our collections and literature under the name of *P. penitalis* Grote. In our catalogues *P. nelumbialis* Smith is listed as a synonym. Upon examination of the genitalia of the specimens in the United States National Museum it became plain to the author that two distinct species were involved. There is a large series reared from *Nelumbo* from various sections of the United States. This is one species and differs markedly in adult and larva from the material reared from corn and Polygonum. At first the writer was inclined to the belief that the name *P. penitalis* might apply to the Polygonum species while *P. nelumbialis* could be retained as a valid specific name for the lotus or *Nelumbo* feeder to which it obviously belongs; but unfortunately Grote described *P. penitalis* from moths reared from larvae feeding in the seed receptacles of the western water lily (*Nelumbo lutea*). An examination of his types at the American Museum of Natural History in New York leaves no doubt that what he described was not the Polygonum species. The name *P. penitalis*, therefore, must be restricted to the true *Nelumbo* feeder and *P. nelumbialis* Smith retained as a synonym. Mr. Ainslie succeeded under artificial conditions in rearing *P. ainsliei* to maturity on *Nelumbo lutea*, the food plant of the true *P. penitalis*, but it is doubtful if both species attack that

plant in nature. The natural food plants of *P. ainsliei* are Polygonum, ragweed, and similar plants; and it is frequently found in corn associated with *P. nubilalis*, for which its larva is easily mistaken.

In fact it is impossible to separate the two on superficial characters, and in structure they so closely resemble each other that a careful microscopic examination is necessary to determine which is which. There is a slight difference in the size of the heads of the mature larvæ. That of *P. nubilalis* is slightly larger, as shown by the drawings (Pl. 10, A, G); but this character is comparative and impracticable for purposes of distinction, since it is necessary to have specimens of both species of the same stage of development for comparison and to be certain at the same time of their instars, something that is rarely possible. The shape of the anal plate used by Miss Mosher is unreliable, both species exhibiting the same forms and the same amount of variation. The clear spots indicating certain muscle attachments on the abdomen are scarcely more reliable. The character is extremely elusive and subject to enough modification to leave one in doubt except with most typical specimens. There seems to be only one reliable character—namely, the arrangement of the setæ and puncture of the anterior epicranial group. In *P. nubilalis*, as mentioned, A<sup>2</sup> is approximate to A<sup>1</sup>, and A<sup>2</sup>, A<sup>1</sup>, and the puncture A<sup>3</sup> are in a straight line or with the puncture postero-ventrad of A<sup>2</sup>. In *P. ainsliei* A<sup>2</sup> is as near to A<sup>3</sup> as to A<sup>1</sup> (or nearer), the three setæ forming almost a right angle with the puncture A<sup>3</sup> lying postero-dorsad of A<sup>2</sup>, the setæ A<sup>1</sup> and A<sup>2</sup> and the puncture forming an obtuse angle. There is some variation in the degree of distance separating A<sup>1</sup> and A<sup>2</sup> in individual specimens and some asymmetry, especially in *P. nubilalis*; but the character seems to hold, and it has been found sufficiently constant through large series to enable accurate determination of all larvæ so far submitted for identification. The pupa is easily distinguished by the front, which is developed into a knob-like projection (Pl. 9, A). Otherwise it is much like that of *P. nubilalis*, though as a rule smaller and a trifle more slender. The average length is 12 to 14 mm.

#### PYRAUSTA PENITALIS

- Pyrausta penitalis* Grote, 1876, in Canad. Ent., v. 8, p. 98; Dyar, List No. Amer. Lepidop., p. 391, no. 4439. 1922  
*Pyrausta nelumbialis* Smith, 1892, in Ent. Amer., v. 6, p. 89; Dyar, List No. Amer. Lepidop., p. 391, no. 4439. 1922

#### ADULT

The adult of this species, especially the female, resembles *P. nubilalis* more closely in superficial characters than does *P. ainsliei*. In fresh specimens the darker shadings have a more distinctly ferruginous tint. As in *P. nubilalis*, there is considerable variation among both males and females, and the males have the same sex scaling at the base of the inner margin of the forewing; but the darkest male of *P. penitalis* is never quite so dark as a pale unrudded specimen of *P. nubilalis*. The hindwing is

pale at the base, and the pale areas bordering the transverse antemedial and transverse postmedial lines of forewing and in the postmedian region of hindwing lack the bright ochreous hue of the male *P. nubilalis*. The yellow is quite as conspicuous in the female, however. Beyond the cell in the forewing of both males and females there is a conspicuous cloudlike blotch of grayish or ferruginous scales, which is much less conspicuous in females of *P. nubilalis* and practically absent in *P. ainshiei*. It is in genitalia characters, however, that the species is most easily and strikingly distinguished. The male genitalia are as figured (Pl. 7, D); apex of tegumen rounded; anellus without anellus lobes but with single, long, stout, ventrally projecting spur (the "calcar" of Pierce)<sup>1</sup>; harpe without spines on inner margin of sacculus; clasper moderate; face of clasper somewhat irregularly oval. Female genitalia (Pl. 8, C, D) without strong chitination anterior to genital opening; chitinated plates posterior to genital opening pear-shaped, tapering anteriorly. Alar expanse of moths, 20 to 36 mm.

#### PUPA

The pupa has a very slightly produced front (Pl. 9, B), not a decided knob like that of *P. ainshiei* but more uneven than that of *P. nubilalis*. The dorsal abdominal spines are nearly obsolete and scarcely distinguishable; abdominal spiracles large, rounded, oval; cremaster very stout and characteristic, broader than long (Pl. 9, C); otherwise as in *P. nubilalis*, except that cephalic end is somewhat more sharply tapering and pupa is generally a trifle stouter; average length, 15 to 16 mm.

#### LARVA

The larva is easily distinguished from that of either *P. nubilalis* or *P. ainshiei*. When full-grown it is 36 to 37 mm. long. The head is considerably larger and the motting of the head different, the darker pigmentation being in the form of groups of small, distinct spots rather than splotches or continuous masses of blackish brown; the ocellar puncture (O<sup>3</sup>) of epicranium lies postero-dorsad of ocellus VI rather than directly posterior to ocellus VI as in *P. nubilalis* and *P. ainshiei*; the mandible (Pl. 11, C) is heavier, oblong rather than square; the median edge is straight or very slightly concave, not outwardly angulated; and the distal tooth concave. Except when the parts are greatly distended the shoulder of the stipes maxillaris has a decided hump which is scarcely perceptible in the other two species (Pl. 11, A, B). Crochets of prolegs are rather stout and more evenly triordinal.

#### DISTINCTIVE CHARACTERS

The three species are very intimately related. In superficial adult characters and in structure of the female genitalia *P. ainshiei* is most readily distinguished. It lacks the sex scaling of the forewing, which is such a prominent character in *P. nubilalis* and *P. penitalis*. On the other hand *P. ainshiei* and *P. nubilalis* are most alike in structure of the male genitalia and hardly separable in larvae, while *P. penitalis* is readily distinguishable from the other two in both. The adult male of *P. nubilalis* is easily distinguished from all American species of *Pyrausta* by its dark, smoky, fuscous forewings and hindwings combined with the distinctly yellow color of the lighter areas.

<sup>1</sup> Pierce, F. N., THE GENITALIA OF THE BRITISH GEOMETRIDAE. 88 p., 48 pl. Liverpool, 1914.

The following tables give the distinguishing structural characters separating the three species:

## ADULTS

## MALE GENITALIA CHARACTERS

1. Anellus consisting of basal plate (juxta) with single, stout, ventrally projecting spur (calcar)..... *P. penitalis*.  
    Anellus consisting of basal plate (juxta) with two long, slender, dorsally projecting arms (anellus lobes) surrounding aedeagus..... 2.
2. Extremity of tegumen rounded..... *P. ainsliei*.  
    Extremity of tegumen trifurcate..... *P. nubilalis*.

## FEMALE GENITALIA CHARACTERS

1. Genital opening strongly chitinated anteriorly..... *P. ainsliei*.  
    Genital opening not strongly chitinated anteriorly..... 2.
2. Chitinated plates posterior to genital opening decidedly pear-shaped viewed from below..... *P. penitalis*.  
    Chitinated plates posterior to genital opening nearly square viewed from below..... *P. nubilalis*.

## PUPÆ

1. Front evenly rounded or with only slight hump..... 2.  
    Front forming prominent projecting knob..... *P. ainsliei*.
2. Cremaster broader than long..... *P. penitalis*.  
    Cremaster longer than broad..... *P. nubilalis*.

## LARVÆ

1. Epicranial setæ and puncture A<sup>1</sup>, A<sup>2</sup>, and A<sup>3</sup> lying in a straight line or with A<sup>3</sup> somewhat postero-laterad of A<sup>2</sup>, not postero-dorsad..... 2.  
    Epicranial setæ and puncture A<sup>1</sup>, A<sup>2</sup>, and A<sup>3</sup> forming an obtuse angle with A<sup>3</sup> postero-dorsad of A<sup>2</sup>..... *P. ainsliei*.
2. Epicranial puncture O<sup>3</sup> lying postero-dorsad of ocellus VI; mandible longer than broad; distal tooth concave..... *P. penitalis*.  
    Epicranial puncture O<sup>3</sup> lying directly posterior to ocellus VI; mandible square; distal tooth pointed..... *P. nubilalis*.



PLATE 7

European corn borer and its nearest American allies:

A.—Male genitalia of *Pyrausta nubilalis*; ventral view of organs spread; aedocagus omitted.

B.—Male genitalia of *P. nubilalis*; aedocagus and penis.

C.—Male genitalia of *P. ainisiei*; ventral view of organs spread; aedocagus omitted.

D.—Male genitalia of *P. penitalis*; ventral view of organs spread; aedocagus omitted.

E.—Forewing venation of *P. nubilalis*; male.

F.—Hindwing venation of *P. nubilalis*; male.

Explanation of symbols applied to male genitalia.

Ae=aedocagus.

An=anellus.

Anl=anellus lobes.

Cl=clasper.

Cn=cornutus (thornlike armature of penis).

Cr=calcar.

Hp=harpe.

J=juxta.

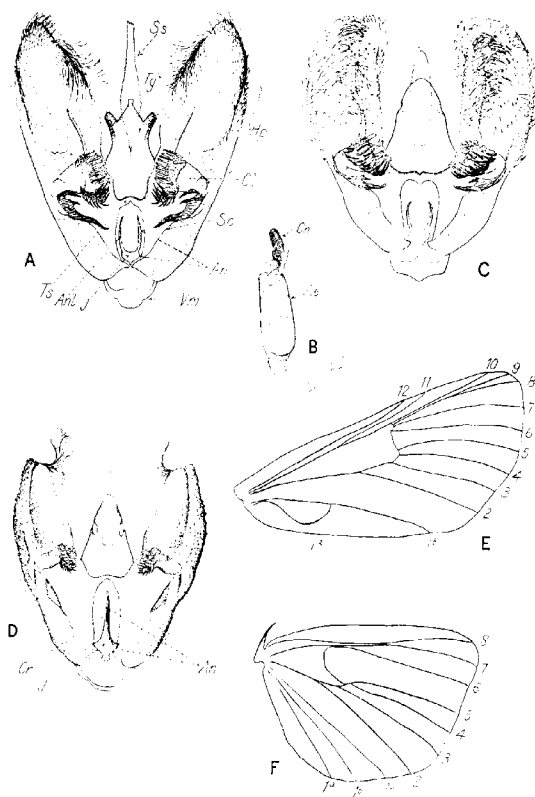
Sc=sacculus of harpe.

Ss=subscaphium.

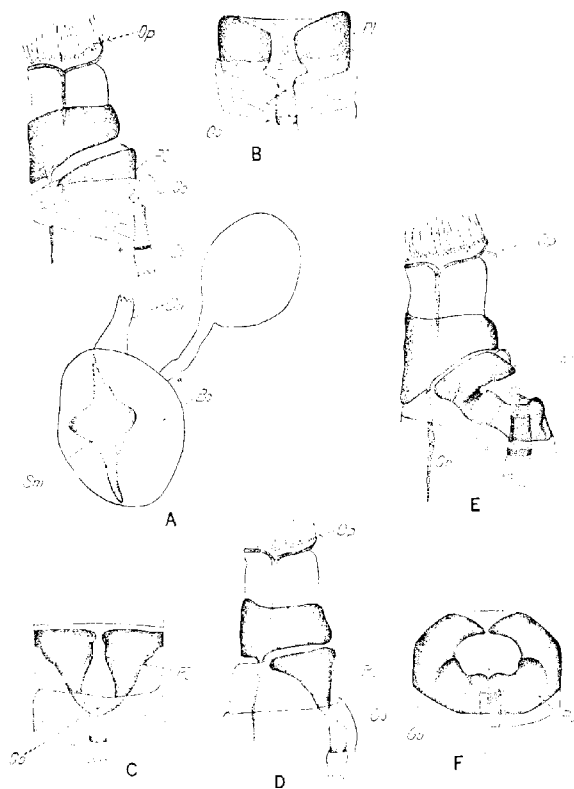
Tg=tegumen.

Ts=transtilla.

Vm=vinculum (sternal portion of ring of the tegumen).







## PLATE 8

### Female genitalia of *Pyrausta* spp.:

- A.—Female genitalia of *P. nubilalis*; lateral view of organs.  
B.—Female genitalia of *P. nubilalis*; ventral view of plates posterior to genital opening.  
C.—Female genitalia of *P. penitalis*; ventral view of plates posterior to genital opening.  
D.—Female genitalia of *P. penitalis*; lateral view of organs.  
E.—Female genitalia of *P. ainislic*; lateral view of organs.  
F.—Female genitalia of *P. ainislic*; ventral view of plates surrounding genital opening.

Explanation of symbols applied to female genitalia.

Bc=bursa copulatrix.

Db=ductus bursae.

Go=genital opening.

Op=ovipositor.

Pl=chitinized plates posterior to genital opening.

Sm=signum (internal armature of bursa).

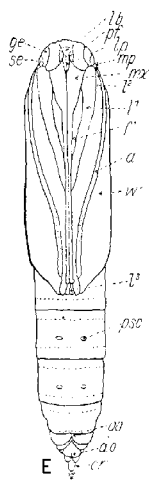
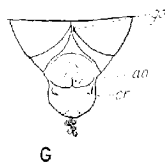
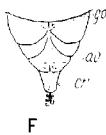
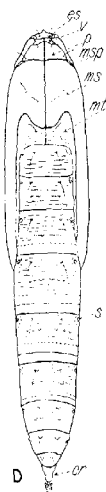
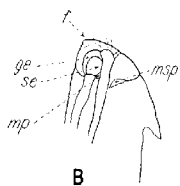
PLATE 9

European corn borer and its nearest American allies:

- A.—Profile of cephalic end of pupa of *Pyrausta nubilalis*.  
B.—Profile of cephalic end of pupa of *P. penitalis*.  
C.—Profile of cephalic end of pupa of *P. nubilalis*.  
D.—Pupa of *P. nubilalis*, female; dorsal view.  
E.—Pupa of *P. nubilalis*, female; ventral view.  
F.—Caudal end of pupa of *P. nubilalis*, female; ventral view.  
G.—Caudal end of pupa of *P. penitalis*, female; ventral view.

Explanation of symbols applied to pupae.

- a=antennae.  
ao=anal opening.  
cr=cremaster.  
es=epicranial suture.  
f=front.  
f<sup>1</sup>=femur of prothoracic leg.  
ge=glazed eye.  
go=genital opening.  
lh=labrum.  
l<sup>1</sup>=prothoracic leg.  
l<sup>2</sup>=mesothoracic leg.  
l<sup>3</sup>=metathoracic leg.  
lp=labial palpi.  
mp=maxillary palpus.  
ms=mesothorax.  
msp=mesothoracic spiracle.  
mt=metathorax.  
mx=maxilla.  
p=prothorax.  
pf=pilifer.  
psc=proleg scar.  
s=abdominal spiracle.  
se=sculptured eye.  
v=vertex.  
w<sup>1</sup>=mesothoracic wing.



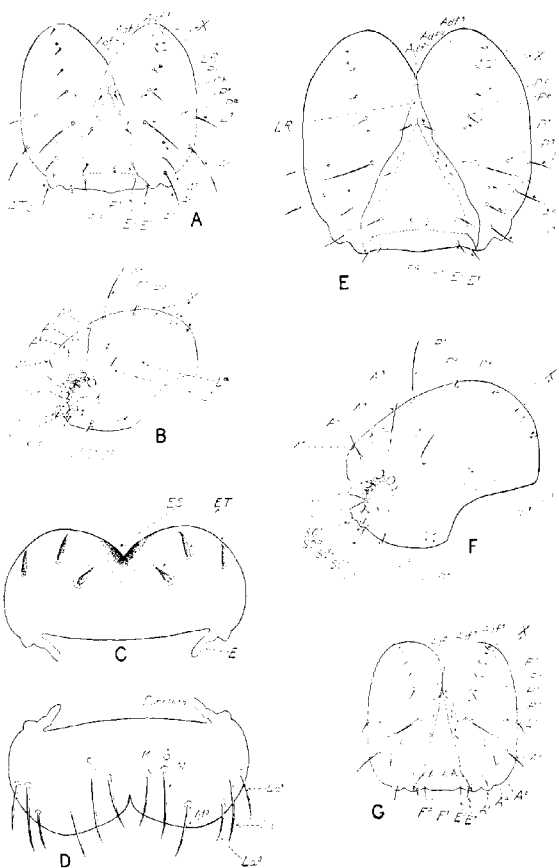


PLATE 10

European corn borer and its nearest American allies:

- A.—Dorsal view of head capsule; larva of *Pyrausta nubilalis*.
- B.—Lateral view of head capsule; larva of *P. nubilalis*.
- C.—Epipharynx; larva of *P. nubilalis*.
- D.—Labrum; larva of *P. nubilalis*.
- E.—Dorsal view of head capsule; larva of *P. penitalis*.
- F.—Lateral view of head capsule; larva of *P. penitalis*.
- G.—Dorsal view of head capsule; larva of *P. ainisiei*.

Explanation of symbols applied to larvae, Plates 10 and 11.

- A<sup>1</sup>, A<sup>2</sup>, A<sup>3</sup>, A<sup>4</sup>=setæ and puncture of anterior group of epicranium.
- Ad<sup>1</sup>, Ad<sup>2</sup>, Ad<sup>3</sup>=adfrontal setæ and puncture of epicranium.
- ATS=antennal shield formed by projection on dorsal surface of epicranium.
- C=cardo.
- E<sup>1</sup>, E<sup>2</sup>=epistomal setæ.
- ER=epipharyngeal rod.
- ES=epipharyngeal shield.
- ET=epipharyngeal setæ.
- Fl, F<sup>1</sup>=frontal seta and puncture.
- G<sup>1</sup>, G<sup>2</sup>=genal seta and puncture.
- L<sup>1</sup>, L<sup>2</sup>=seta and puncture of lateral group of epicranium.
- La<sup>1</sup>, La<sup>2</sup>, La<sup>3</sup>=lateral group of setæ of labrum.
- LR=longitudinal ridge of epicranium.
- M<sup>1</sup>, M<sup>2</sup>, M<sup>3</sup>=median group of setæ of labrum.
- nt=thornlike chitinization on proleg bearing abdominal segments.
- O<sup>1</sup>, O<sup>2</sup>, O<sup>3</sup>, O<sup>4</sup>=setæ and puncture of ocellar group of epicranium.
- P<sup>1</sup>, P<sup>2</sup>, P<sup>3</sup>, P<sup>4</sup>=setæ and punctures of posterior group of epicranium.
- SO<sup>1</sup>, SO<sup>2</sup>, SO<sup>3</sup>, SO<sup>4</sup>=setæ and puncture of subocellar group of epicranium.
- X=ultraposterior setæ and puncture of epicranium.

PLATE 11

European corn borer and its nearest American allies:

- A.—Labium and maxillæ; larva of *Pyrausta nubilalis*.
- B.—Left maxilla; larva of *P. penitalis*.
- C.—Mandible; larva of *P. penitalis*.
- D.—Mandible; larva of *P. nubilalis*.
- E.—Character of skin granulations, highly magnified; larva of *P. nubilalis*.
- F.—Second thoracic and eighth and ninth abdominal segments of larva of *P. nubilalis*, showing granulose areas above seta VII.
- G.—Setal map of prothoracic, mesothoracic, third, eighth, and ninth abdominal segments; larva of *P. nubilalis*.
- H.—Crochet arrangement; abdominal proleg; larva of *P. nubilalis*.

